

Clarke, Mo

COLOMBIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 10-098518-A-00006-0000

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P-3675

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
E. S. D.

Re: Expediente No. 10-098.518A
Solicitud de patente a nombre de REATA PHARMACEUTICALS,
INC. Y TRUSTEES OF DARTMOUTH COLLEGE

Yo, SILVANA MARIA LOPEZ DÍAZ, identificada como aparece al pie de mi firma, domiciliada en la carrera 11 A No. 86-53, piso 6, y actuando como apoderada sustituta de acuerdo con la sustitución adjunta de la sociedad PHARMACEUTICALS, INC. AND TRUSTEES OF DARTMOUTH COLLEGE, domiciliada en Estados Unidos de América, solicitante de la patente de la referencia, interpongo recurso de reposición contra la resolución No. 55542 emitida el 18 de septiembre de 2013 por el Superintendente de Industria y Comercio, por medio de la cual se niega el privilegio de patente de invención para la solicitud contenida en el expediente de la referencia.

Mi mandante permite manifestar a ese despacho que se reafirma en todos y cada uno de los argumentos que hacen parte integrante de la respuesta al oficio No. 6056 notificado por fijación en lista el 9 de abril de 2013, presentada ante ese despacho el 8 de agosto de 2013.

1. FUNDAMENTOS DEL RECURSO

1.1 La indebida interpretación del artículo 18 de la Decisión 486/00.

Es deseo del solicitante presentar argumentos contra las declaraciones de esa Superintendencia indicando como primera medida que el examinador no realizó una adecuada interpretación del artículo 18 de la Decisión 486/00 y parte de premisas erradas, en cuanto a la supuesta obviedad de la materia objeto de la presente invención, y realiza un equivocado análisis del requisito en cuestión, para finalmente negar sin justificación la patente para la solicitud en comento.

En este sentido, volvemos sobre el particular que el artículo 18 de la Decisión 486/00 determina 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”*. (El subrayado es nuestro).

Lo anterior significa que el análisis de nivel inventivo **NO** es una comparación de los objetos de las anterioridades y la solicitud en estudio. El análisis de nivel inventivo **debe demostrar** la obviedad de la invención y establecer cómo ésta no contiene un salto cualitativo, que demuestre su altura inventiva, teniendo en cuenta que se hace **necesario demostrar** que la invención analizada carece de nivel inventivo y hubiese sido obvia para una persona versada en la materia.

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En vista de lo anterior, el solicitante desea se tengan en cuenta los siguientes argumentos así como los argumentos inicialmente presentados en respuesta al oficio No. 6056 notificado por fijación en lista el 9 de abril de 2013, los cuales dan soporte a los dichos siguientes argumentos y demuestran que la solicitud de la referencia cumple con el requisito de nivel inventivo.

El solicitante muy respetuosamente desea expresar que la presente invención tiene nivel inventivo y no es en absoluto obvio a la luz de los documentos D1 y D2, teniendo en cuenta que la forma polimórfica descrita en la reivindicación 1 posee una combinación de propiedades ventajosas que demuestran su nivel superior con respecto a las formas descritas en el estado del arte.

En primer lugar, es deseo del solicitante resaltar que la forma sólida B de RTA-402 (methy1-2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid; CDDO-Me) de la presente invención **presenta inesperadamente una alta estabilidad térmica y mecánica** (ver US2009/0048204; párrafo 183; página 15, tabla 9), contrario a lo esperado para compuestos de forma amorfa según lo descrito en D2 (ver parte superior de la columna derecha y parte inferior en la columna izquierda en la página 253). Cabe resaltar que el documento US2009/0048204 fue publicado el 19 de febrero de 2009, por lo que no es considerado dentro del estado de la técnica de la presente invención.

Adicionalmente, el solicitante muy respetuosamente reitera lo indicado en respuesta al oficio No. 6056 notificado por fijación en lista el 9 de abril de 2013, respecto a que la forma sólida B de la presente invención **presenta una mayor biodisponibilidad que otras formas de CDDO-Me**. A este respecto, el solicitante presenta como argumento lo expuesto en la solicitud PCT WO2010/093944 con una fecha de prioridad de un día después de la prioridad de la presente solicitud, revelando que 30 mg de la forma B presentan casi los mismos valores de área-bajo-la-curva (AUC) que 150 mg de la forma A (ver página 12). De esta forma, se demuestra que **la forma sólida B de la presente solicitud tiene una biodisponibilidad aproximadamente cinco veces mayor que la biodisponibilidad de la forma A sugerida según el examinador en los documentos D1 y D2**. Lo anterior se complementa con los resultados incluidos en el artículo de Kim et al. (2008) presentado como anexo el 8 de agosto de 2013 en la respuesta al oficio No. 6056, donde se reporta que la atorvastatina amorfa mejora solo de 2 a 3 veces la biodisponibilidad de la forma cristalina.

Así mismo y adicional a los argumentos anteriormente presentados, es deseo del solicitante indicar que la materia reclamada en las reivindicaciones 6, 8 y 9 de la presente solicitud debe ser considerada como inventiva, teniendo en cuenta que según el documento US2009/0048204 **las formulaciones de copolímero de ácido metacrílico y hidroxipropil-metil-celulosa muestran una mayor biodisponibilidad en relación a la forma pura de CDDO-Me** (ver página 12, párrafo 219 y página 18, tabla 22).

Igualmente, es deseo del solicitante hacer referencia a la página 339 del artículo "*Drug polymorphism and dosage form design: a practical perspective*" (Singhal y Curatolo, 2004), considerado dentro del estado de la técnica y que reporta que "*la falta de información sobre los medicamentos amorfos puede ser debido a la inestabilidad física y química de estas formas, lo que*

lleva a la resistencia en el desarrollo de una forma amorfa en un producto comercial de la droga. Por lo tanto, una evaluación de las propiedades mecánicas de los fármacos amorfos no se investiga de forma rutinaria en la industria farmacéutica” (ver página 339, columna superior izquierda). Teniendo en cuenta esta afirmación, el solicitante desea indicar que el examinador no está considerando la información consignada en el estado de la técnica que podría motivar a una persona del oficio normalmente versada en la materia a NO intentar hacer una forma amorfa de CDDO-Me.

Copias de los documentos citados en la anterior argumentación se encuentran relacionadas en el anexo 1.

En consecuencia, teniendo en cuenta lo anterior y los argumentos indicados el respuesta al oficio anteriormente mencionado, es claro que la composición farmacéutica que comprende la forma B de RTA-402 reclamada en la presente solicitud en las reivindicaciones 1 a 10 debe ser considerada como inventiva, ya que presenta un efecto técnico inesperado que no sería obviamente deducido por la persona con conocimientos en la técnica a partir de las enseñanzas descritas en D1 y D2.

Así las cosas, tenemos que, al basarse el examinador en la información de la solicitud en estudio y al reiterar que las características técnicas de la invención se derivaban de manera obvia de D1 y D2 sin proporcionar argumentos adicionales que demostraran tales conclusiones, queda claro que se está haciendo un estudio retrospectivo, dado que se coloca a sí mismo en el momento en el cual la solicitud de patente ya ha resuelto el problema técnico existente, y no toma en cuenta el marco técnico que existía al momento de iniciar el desarrollo del objeto de patentabilidad de la solicitud en referencia, ni tampoco hace el esfuerzo intelectual de ubicarse en el momento de la presentación de la solicitud prioritaria, con el fin de llevar a cabo un análisis adecuado del requisito de patentabilidad en cuestión.

Por otra parte, teniendo en cuenta el documento emitido por la Superintendencia de Industria y Comercio que permite establecer la manera como debe realizarse el examen de nivel inventivo (INSTRUCTIVO DE EXAMEN DE SOLICITUDES DE PATENTES DE INVENCION Y MODELO DE UTILIDAD), en donde se establece que “Es decir, el estado de la técnica se debe examinar sin el beneficio del conocimiento ofrecido retrospectivamente por la invención reivindicada. La indicación o la sugerencia que permita llegar a la invención reivindicada deben proceder del estado de la técnica o de los conocimientos generales de la persona del oficio normalmente versada en la materia, y no basarse en la divulgación del solicitante” (subrayado fuera de texto).

Adicionalmente, el Tribunal Andino de Justicia, en el proceso 12-IP-98, ha establecido que “con el requisito del nivel inventivo, lo que se pretende es dotar al examinador técnico de un elemento que le permita afirmar o no si a la invención objeto de estudio no se habría podido llegar a partir de los conocimientos técnicos que existían en ese momento dentro del estado de la técnica (...). En este punto conviene advertir que uno es el examen que realiza el técnico medio respecto de la novedad y otro el que se efectúa con respecto al nivel inventivo; si bien en uno y otro se utiliza como parámetro de referencia el ‘estado de la técnica’, en el primero, se coteja la invención con las ‘anterioridades’ existentes dentro de aquella, cada uno (sic) por separado, mientras que en el segundo (nivel inventivo) se exige que el técnico medio que realiza el examen debe partir del conocimiento general

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que él tiene sobre el estado de la técnica y realizar el cotejo comparativo con su apreciación de conjunto, determinando si con tales conocimientos técnicos existentes ha podido o no producirse tal invención". (Subrayado y negrilla fuera del texto).

Tenemos entonces como la jurisprudencia también hace énfasis sobre el hecho que el nivel inventivo de una solicitud de patente debe ser evaluado teniendo en cuenta la información que existía en el momento en que la invención correspondiente no era conocida. En este caso, por los aspectos técnicos mencionados con anterioridad, el examinador realizó un análisis partiendo de la base provista por los conocimientos ofrecidos precisamente por la solicitud de patente objeto de negación por parte de la Superintendencia de Industria y Comercio.

Por lo tanto, en este punto se configura una indebida interpretación del artículo 18 de la Decisión 486 de 2000, ya que este establece que *"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"*. Con relación a lo anterior, el hecho de haber realizado un análisis de patentabilidad de la invención tipo hindsight involucra haber llevado a cabo una indebida interpretación de la norma aplicable (artículo 18 de la Decisión 486 y, en consecuencia, artículo 14 ibídem) al haberle otorgado un alcance mayor a la norma que aquella que en verdad tiene.

Con respecto a este punto, atentamente nos permitimos decir que es claro que era necesario que el examinador realizara una interpretación adecuada de lo dispuesto en la norma citada anteriormente y, sobretodo, lógica de la misma, de tal manera que se considere que el momento que se debe tener en cuenta para determinar el estado de la técnica no es aquel cuando ya el contenido de la patente objeto de solicitud ha sido divulgada sino, precisamente, antes de este.

Como bien lo indica la Superintendencia de Industria y Comercio *"para la evaluación de los requisitos de patentabilidad resulta de la mayor importancia no solo los documentos antecedentes"*, la expresión *"no solo"* significa que además de los documentos, es decir como complemento de ellos, son importantes los conocimientos, la información y experiencia del examinador de patentes. En ningún momento, ninguna norma ni jurisprudencia ha eximido a la Oficina de Patentes de la obligación de aportar pruebas para negar la solicitud. Por el contrario, la Jurisprudencia Andina, el Manual Andino de Patentes, la práctica de la oficina de patentes y ahora el texto citado por la Superintendencia de Industria y Comercio, son claros en decir que la evaluación de la patentabilidad de una solicitud se basa en el establecimiento del estado de la técnica, documentos o pruebas, que permitan corroborar o ser sumados a los conocimientos del examinador para emitir un concepto donde se evidencie la falta de novedad y/o nivel inventivo de la invención.

Los artículos 14 y 18 de la Decisión 486 de la Comunidad Andina de Naciones establecen cuáles son los requisitos de patentabilidad y qué se entiende por nivel inventivo, lo cual quedó ampliamente definido y explicado en los párrafos previos de esta demanda, tanto desde el punto de vista de su contenido como de su evaluación.

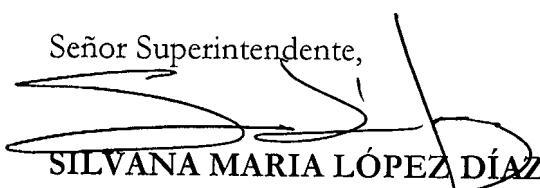
Teniendo en cuenta lo dicho anteriormente, solicito a usted lo siguiente:

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a) Que de conformidad con lo previsto por los artículos 3 (inciso 3); 35 (inciso 2) y 59 (inciso 2) del Código Contencioso Administrativo se consideren y se resuelvan **TODOS** los argumentos y **TODAS** las cuestiones planteadas así como las que aparezcan con motivo de este recurso.

b) Que se revoque la resolución No. **55542** emitida el 18 de septiembre de 2013 expedida por el Superintendente de Industria y Comercio, se declare infundada la oposición presentada por LAFRANCOL S.A., y se proceda a conceder el privilegio de patente de invención a la solicitud de mi mandante

Señor Superintendente,



SILVANA MARIA LÓPEZ DÍAZ

C.C. 22468787 de Barranquilla

T.P 147463 del CSJ

notificaciones@clarkemodet.com.co

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Clarke, Modet & C^o

COLOMBIA

P-3675

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

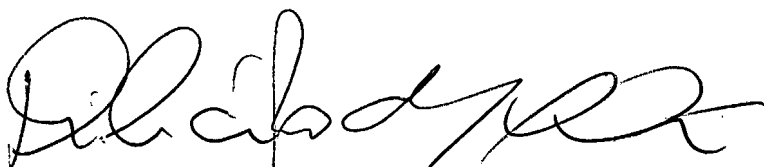
E. S. D.

Re: Expediente No. 10-098.518A

Solicitud de patente a nombre de REATA PHARMACEUTICALS, INC. Y
TRUSTEES OF DARTMOUTH COLLEGE

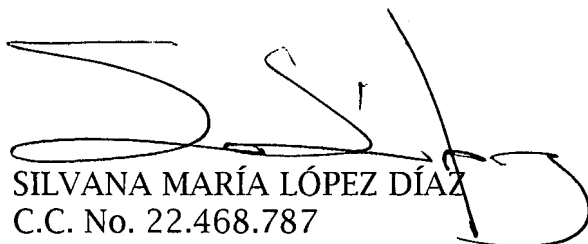
Yo, DILIA RODRÍGUEZ D' ALEMAN, identificada como aparece al pie de mi firma, domiciliada en Bogotá, Colombia, atentamente informo a usted que sustituyo el poder con que obro a la doctora SILVANA MARÍA LÓPEZ DÍAZ, domiciliada en Bogotá, identificada con la Cédula de Ciudadanía No. 22.468.787 y portadora de la tarjeta profesional No. 147.463 del CSJ, con las mismas facultades con que me fueron conferidas para este caso.

Señor Superintendente,



DILIA RODRIGUEZ D' ALEMAN
C.C. 41.654.882 de Bogotá
TP 20824 CSJ

Acepto sustitución



SILVANA MARÍA LÓPEZ DÍAZ
C.C. No. 22.468.787
TP 147463 CSJ

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PRESENTACION PERSONAL
El anterior escrito fue presentado ante el
NOTARIO ONCE (E) DEL CIRCULO DE BOGOTÁ, D.C.
personalmente por D. ALFONSO RODRIGUEZ
quien exhibió la c.c. 4103482 Alfonso
y Tarjeta Profesional No. 2082 C.S.J.
Firma [Signature] 20 SEP 2013
Fecha [Signature]
Nelson J. Sánchez García
NOTARIO (E)



Anexo 1

- Walling J., Parent S.D., Jonaitis D.T., Kral JR. R.M. Novel Forms of CDDO Methyl Ester US2009/0048204 A1.
- Zhang J., Meyer C.J. Delayed Release, Oral Dosage Compositions That Contain Amorphous CDDO-Me. Solicitud PCT WO2010/093944 A2.
- Singhal D. y Curatolo W. (2004) Drug Polymorphism and Dosage Form Design: A practical Perspective. *Advanced Drug Delivery Reviews* 56, 335-347.



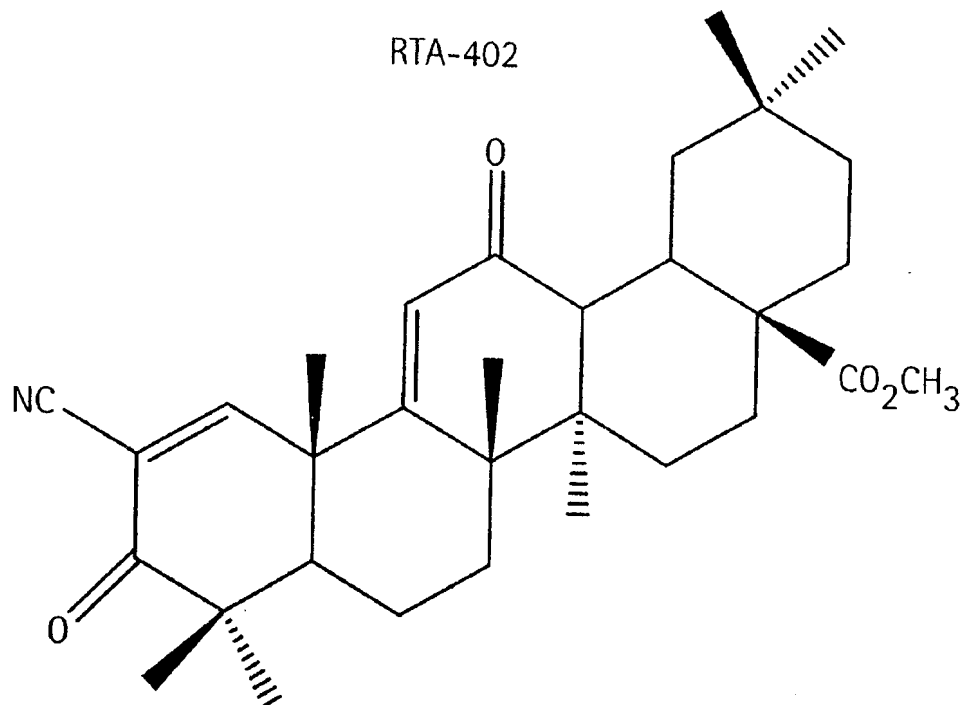
US 20090048204A1

(19) **United States**(12) **Patent Application Publication**
Walling et al.(10) **Pub. No.: US 2009/0048204 A1**(43) **Pub. Date: Feb. 19, 2009**(54) **NOVEL FORMS OF CDDO METHYL ESTER****Publication Classification**(75) **Inventors:** **John Walling**, Irving, TX (US);
Stephan D. Parent, W. Lafayette,
IN (US); **David T. Jonaitis**,
Lafayette, IN (US); **Robert M.**
Kral, JR., Grapevine, TX (US)(51) **Int. Cl.**
A61K 31/215 (2006.01)
C07C 69/753 (2006.01)
A61K 31/7068 (2006.01)
A61P 35/00 (2006.01)

Correspondence Address:

FOLEY AND LARDNER LLP**SUITE 500****3000 K STREET NW****WASHINGTON, DC 20007 (US)**(52) **U.S. Cl.** **514/49; 560/116; 514/529**(73) **Assignee:** **Reata Pharmaceuticals, Inc.**(21) **Appl. No.:** **12/191,176**(22) **Filed:** **Aug. 13, 2008****Related U.S. Application Data**(60) **Provisional application No. 60/955,939, filed on Aug.**
15, 2007.(57) **ABSTRACT**

A triterpenoid compound, methyl 2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oate (CDDO methyl ester), has a non-crystalline, glassy solid form and a non-hydrous crystalline form that can prepared, for example, from a saturated methanol solution. The glassy form displays an enhanced bioavailability over the non-hydrous crystalline form. Each form of CDDO methyl ester is a superior candidate for use, typically in solid dosage form, for treating a variety of disease states, generally associated with inflammation.



$C_{32}H_{43}NO_4$
Mol. Wt.: 505.69

FIG. 1

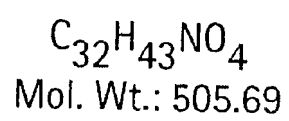
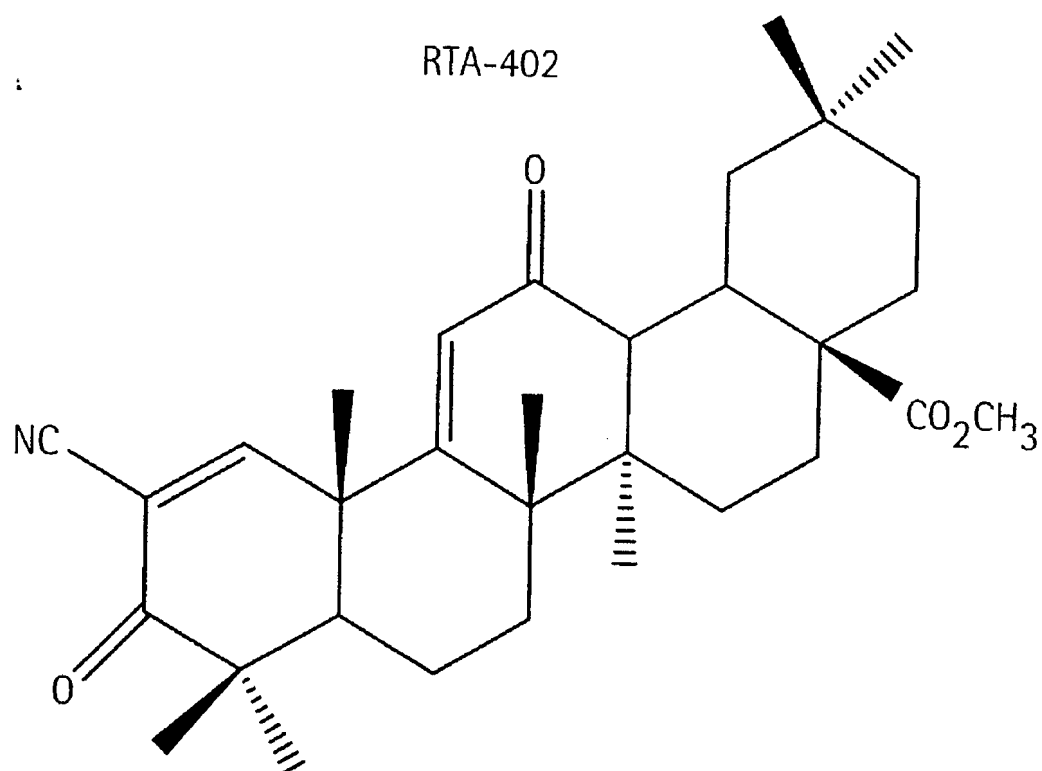
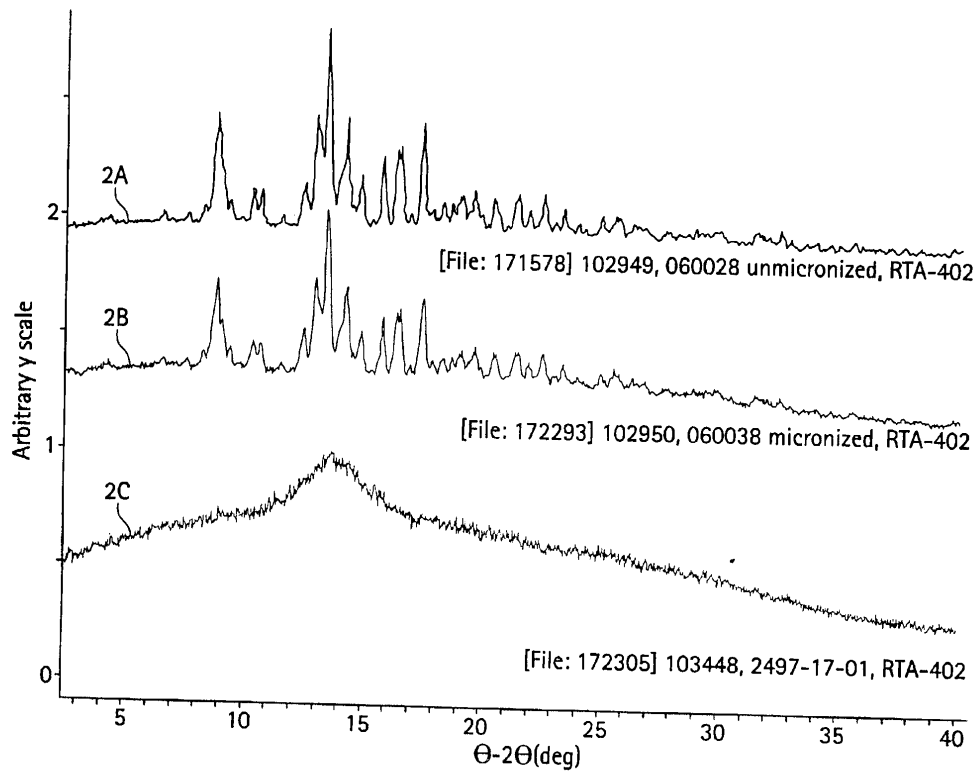
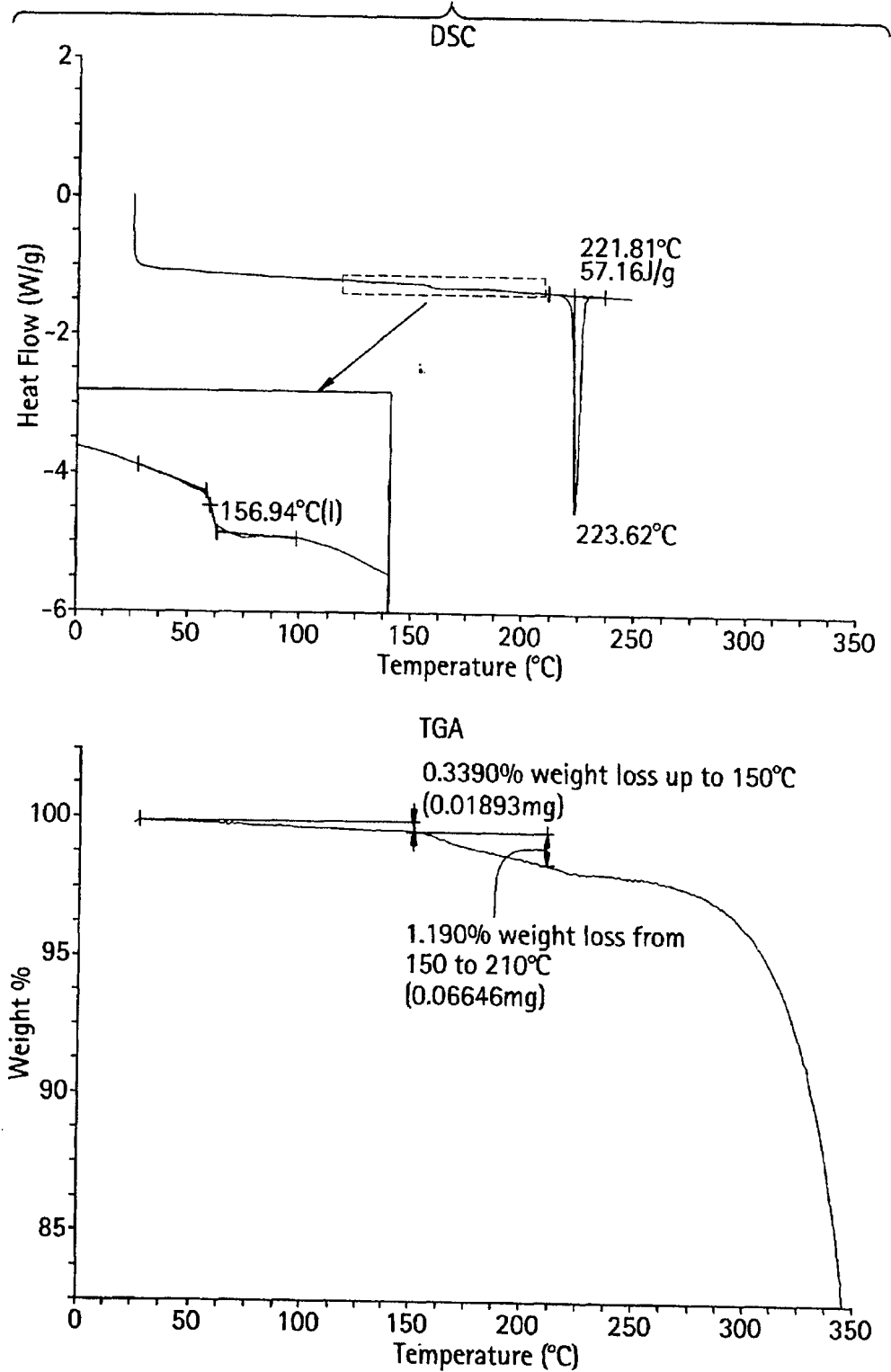


FIG. 2



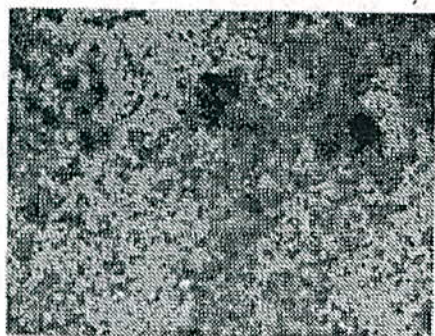
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FIG. 3

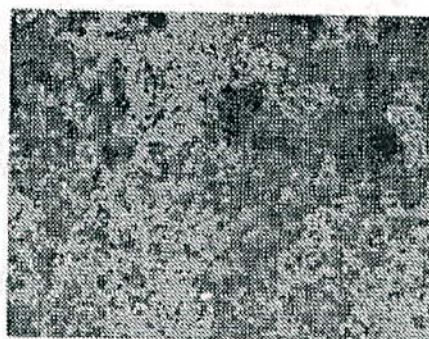


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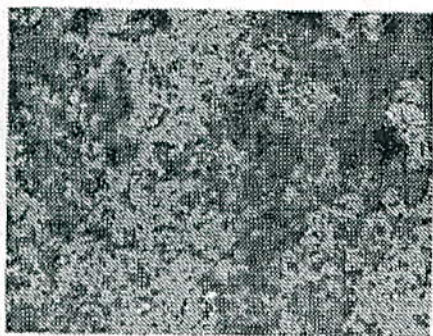
FIG. 4



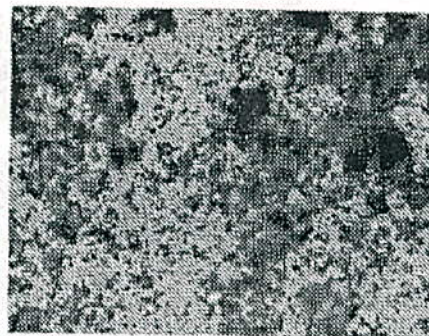
28.9°C



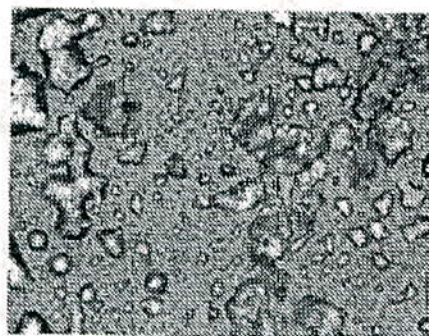
150.1°C



176.4°C



200.2°C



225.9°C

FIG. 5

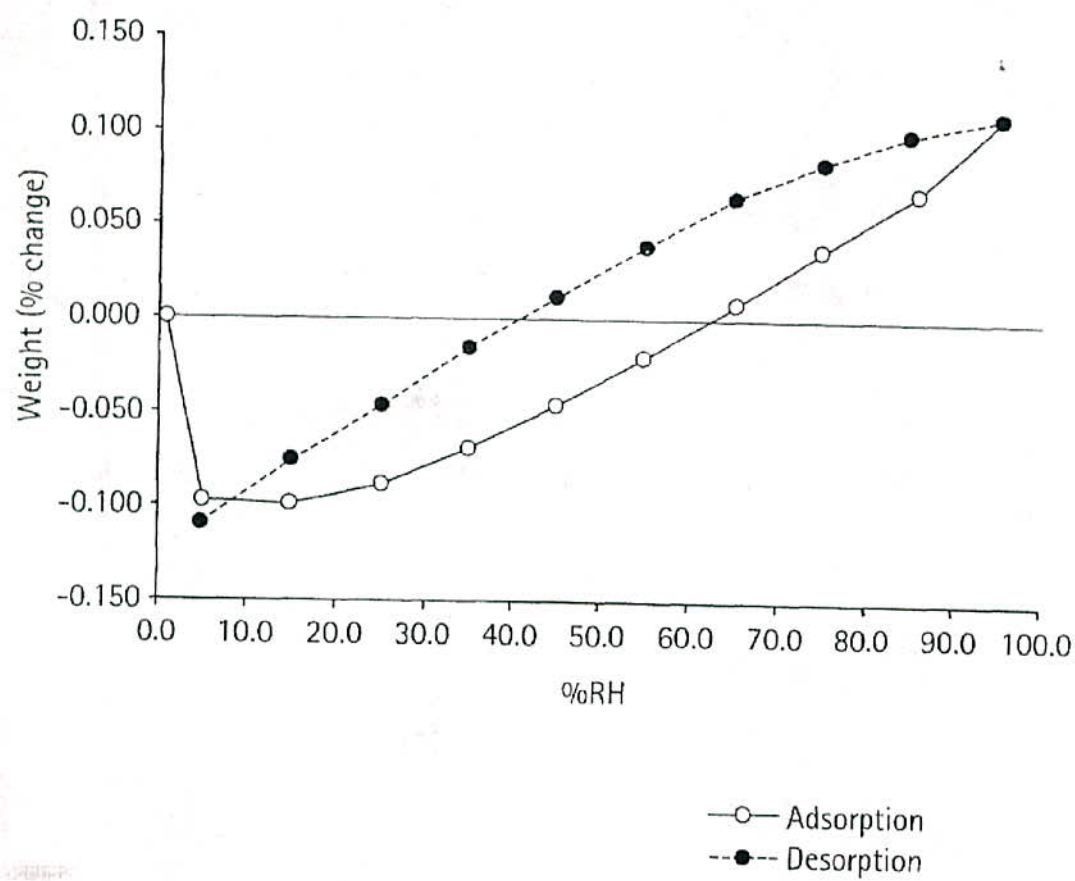
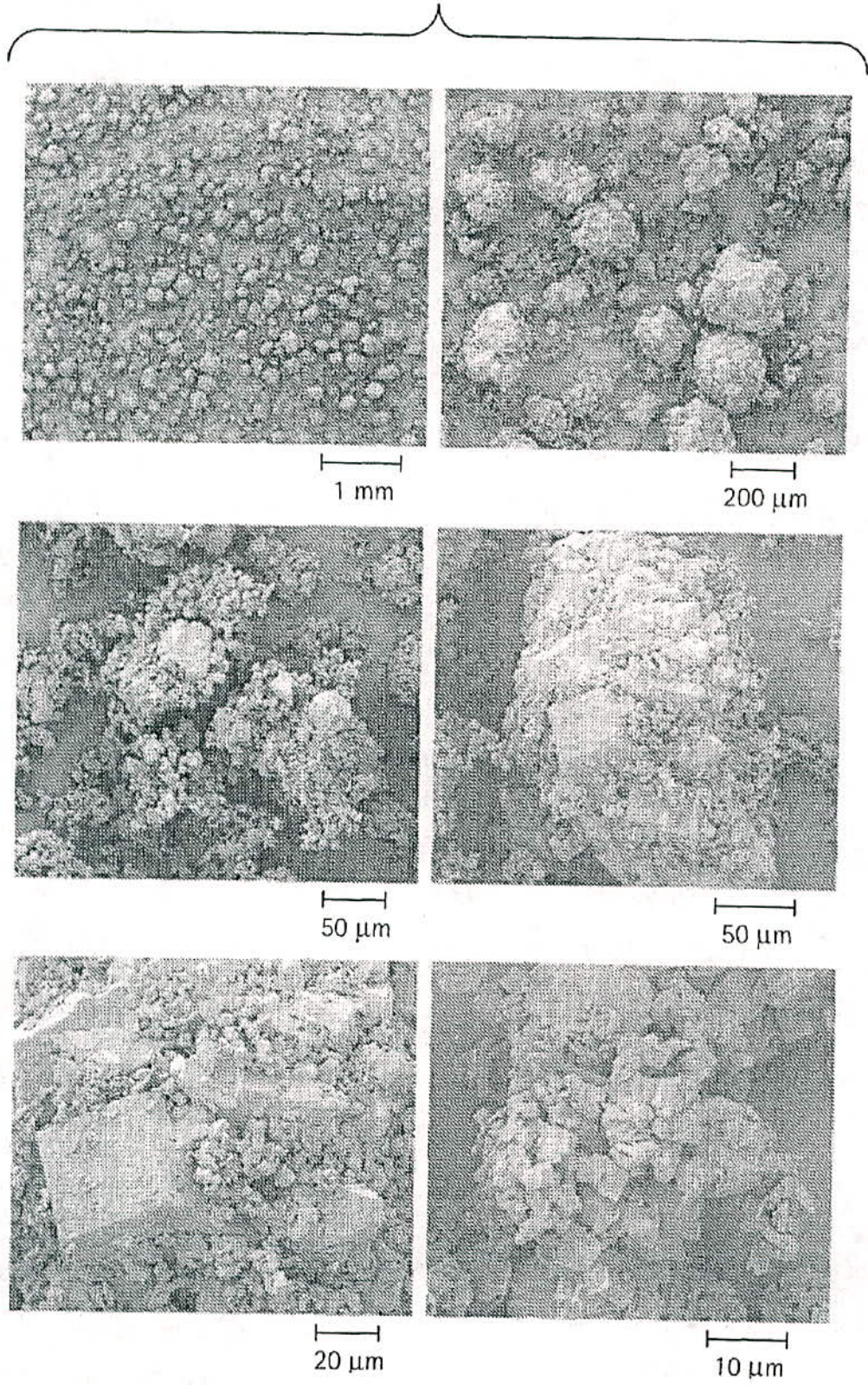


FIG. 6



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FIG. 7

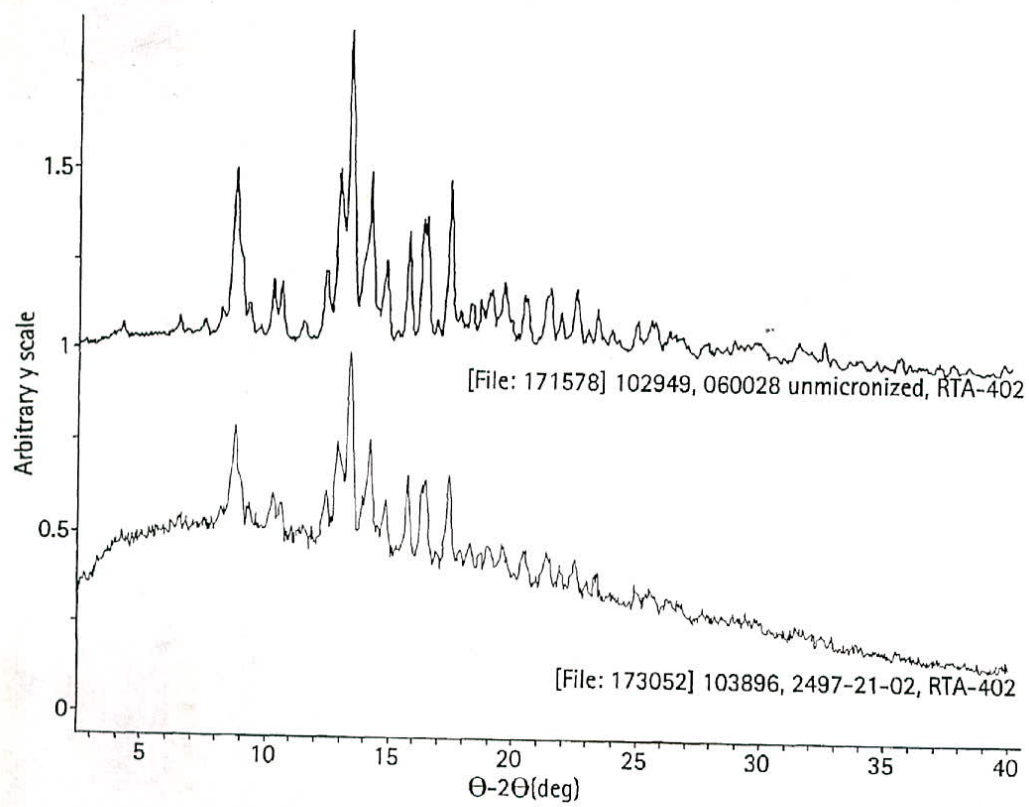
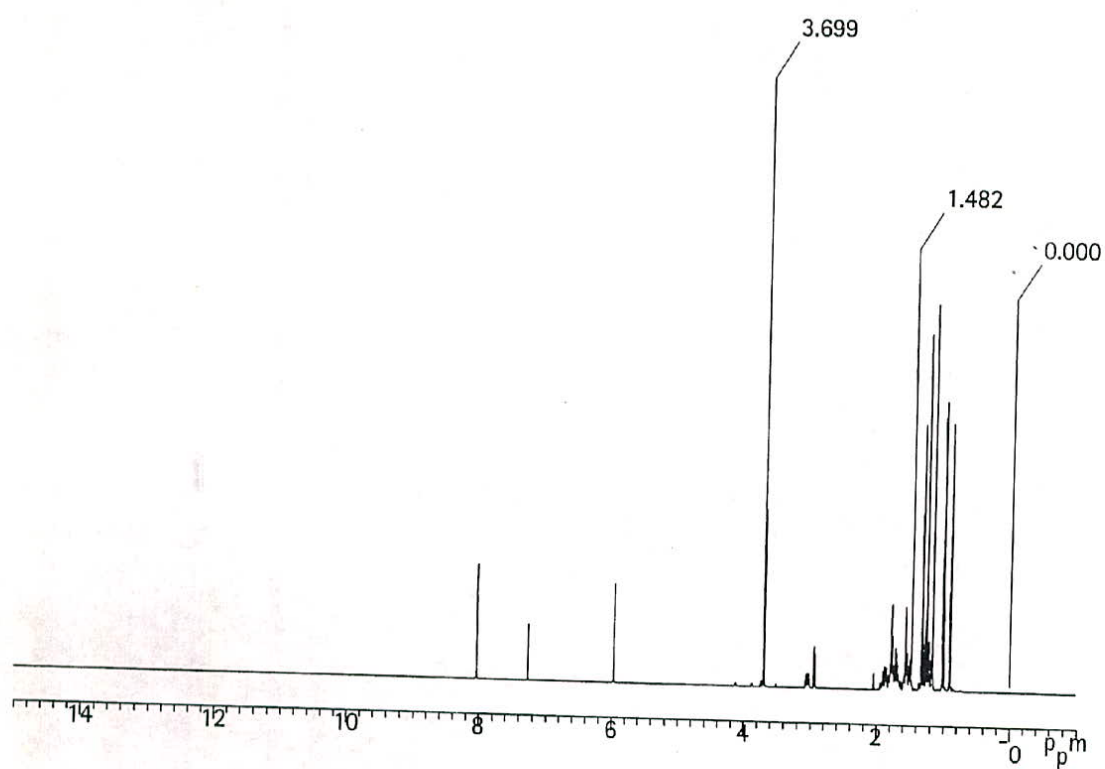


FIG. 8



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FIG. 9

DSC

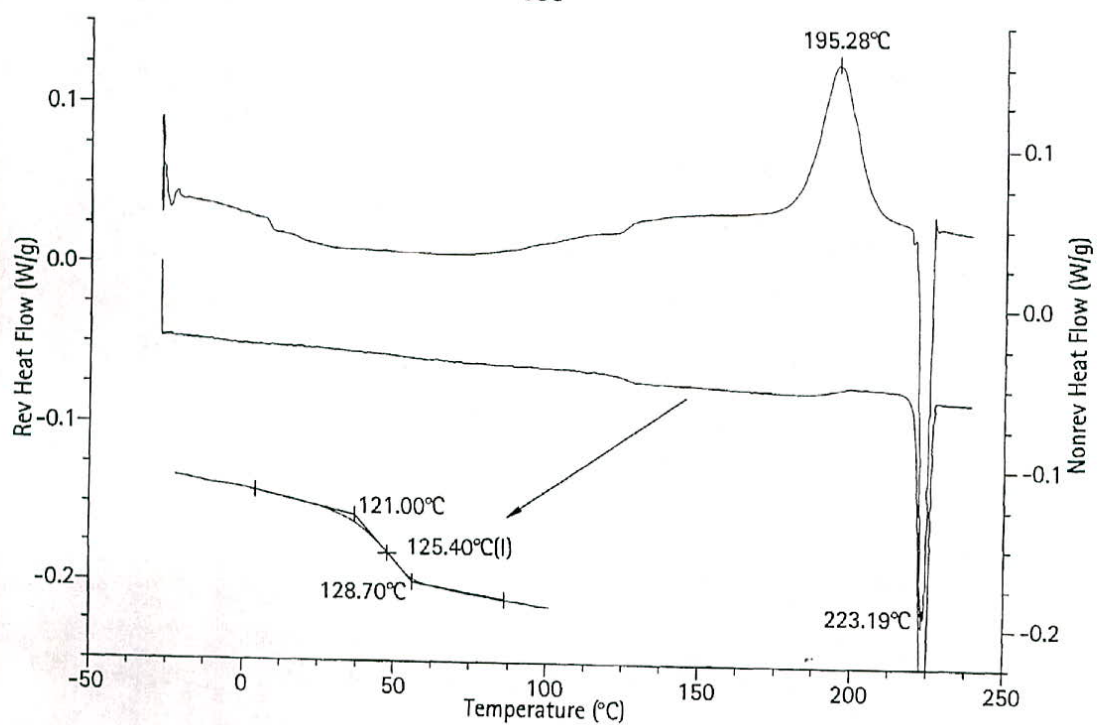
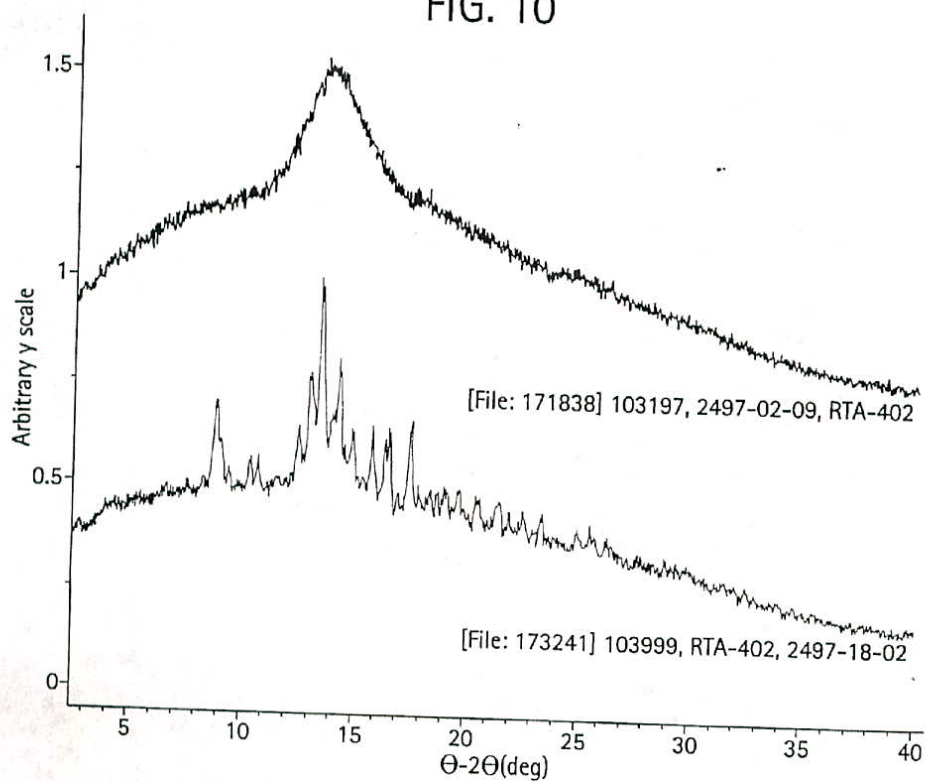


FIG. 10



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FIG. 12

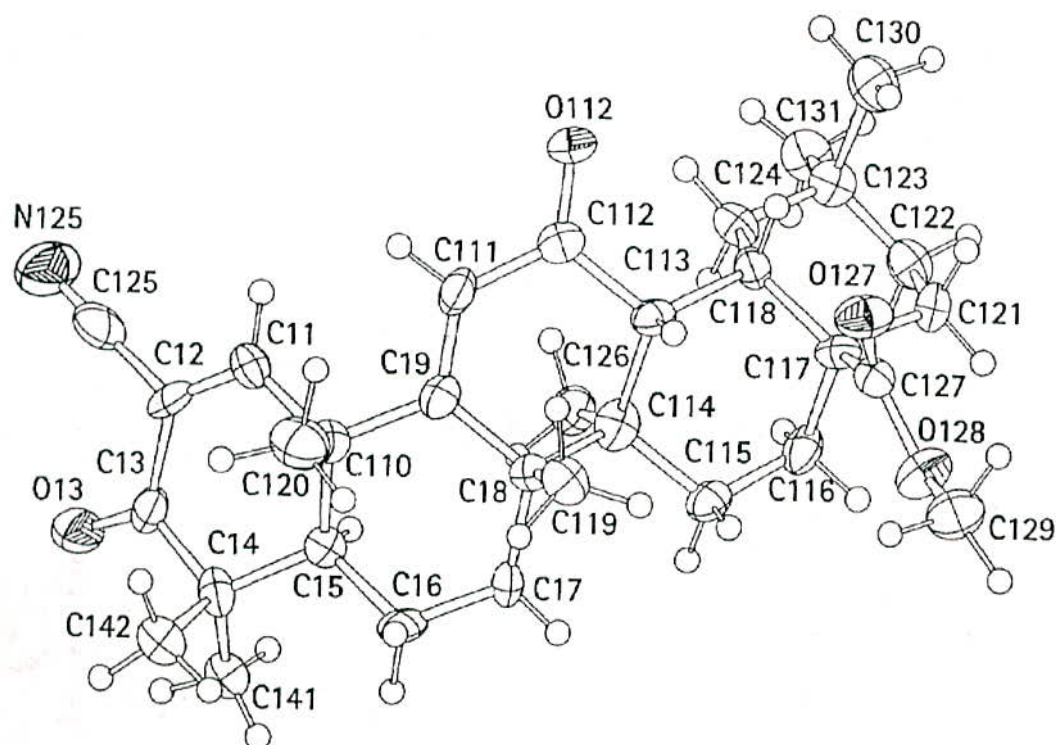


FIG. 13

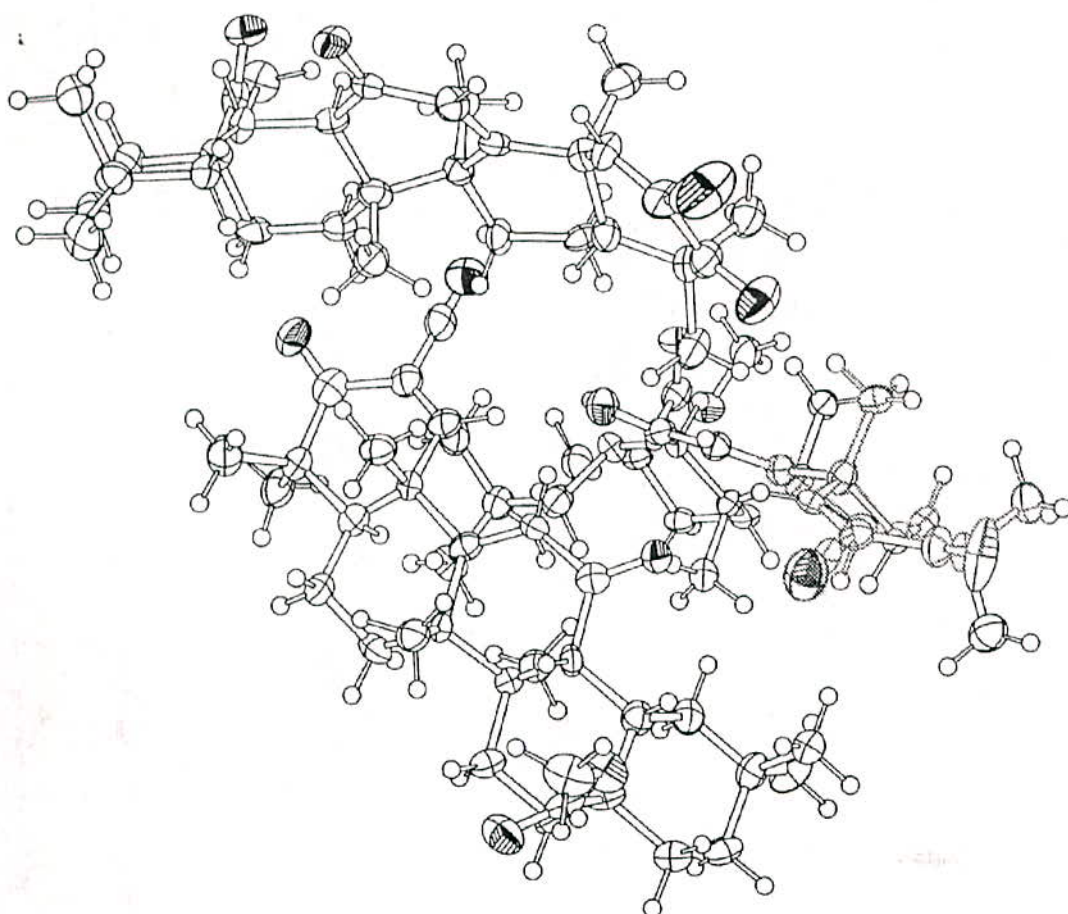


FIG. 14

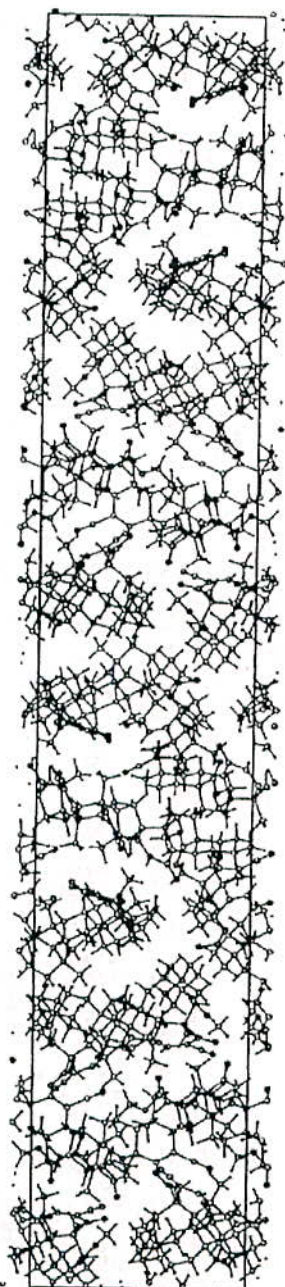
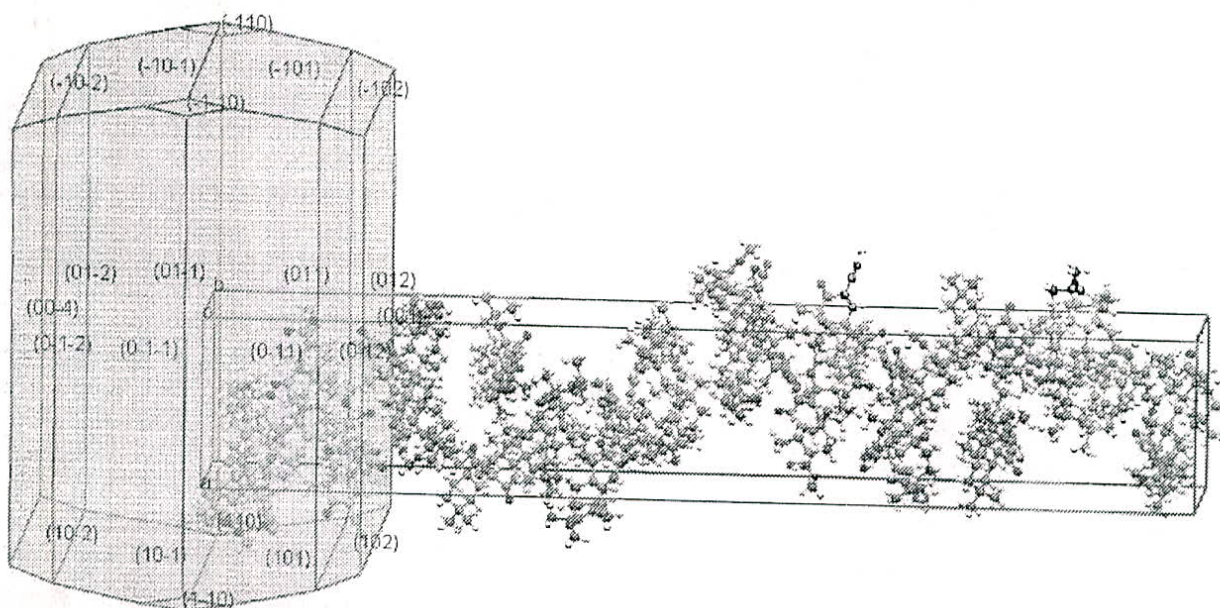


FIG. 15



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FIG. 16

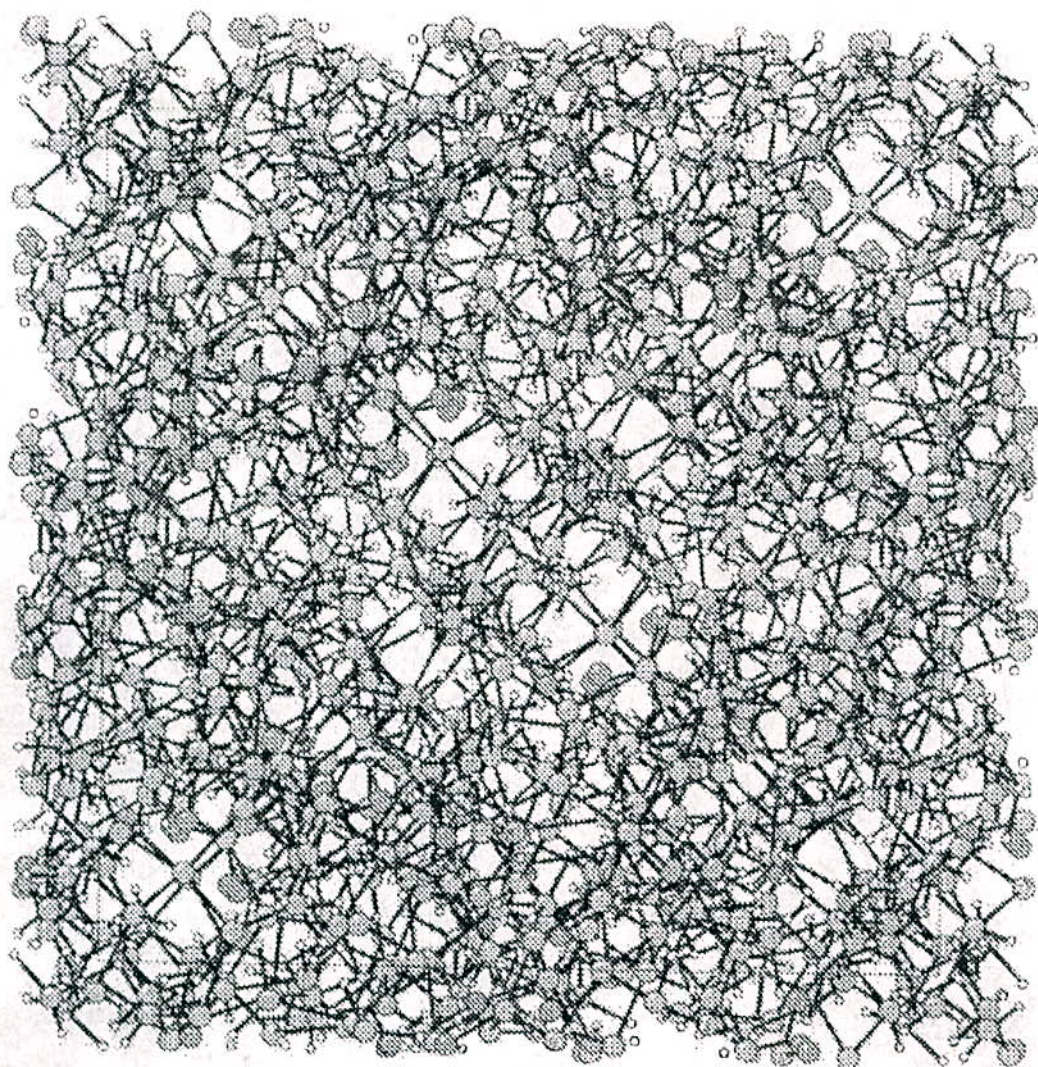
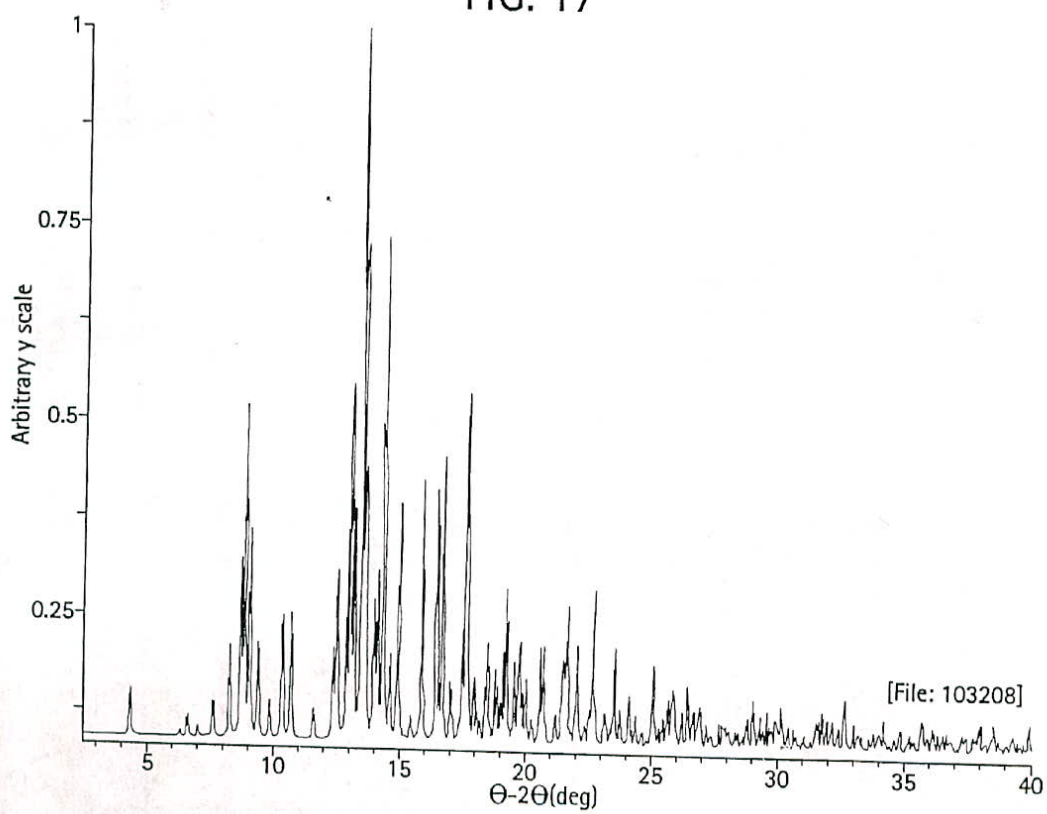


FIG. 17



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FIG. 18

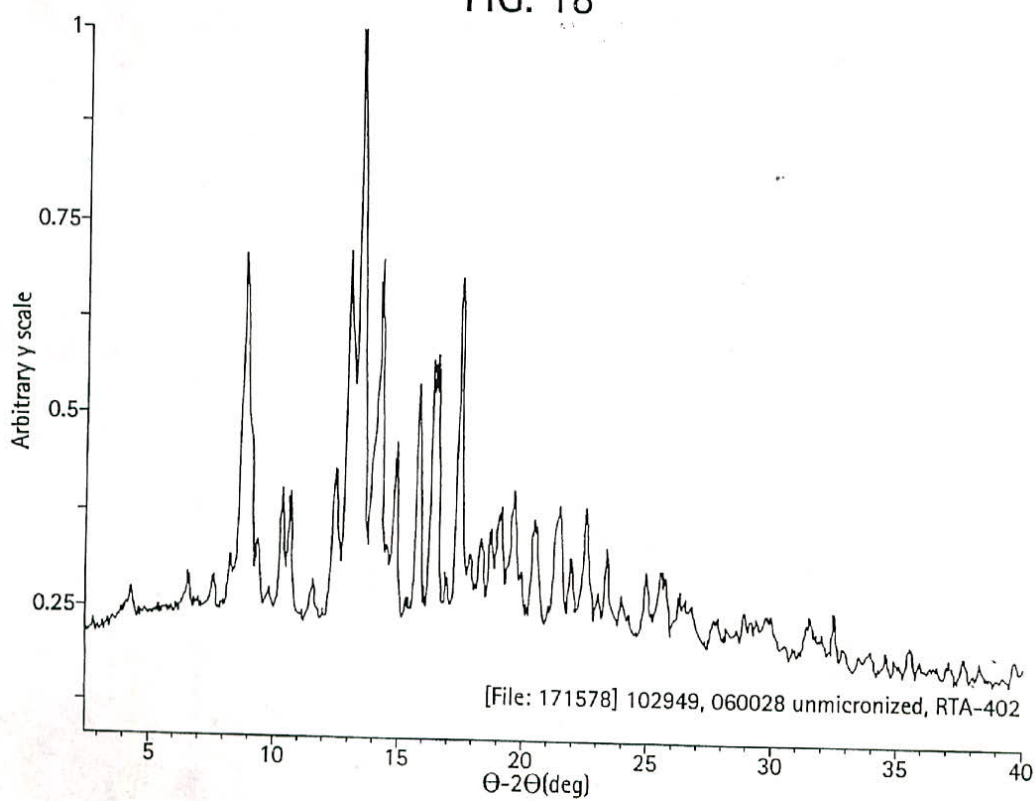
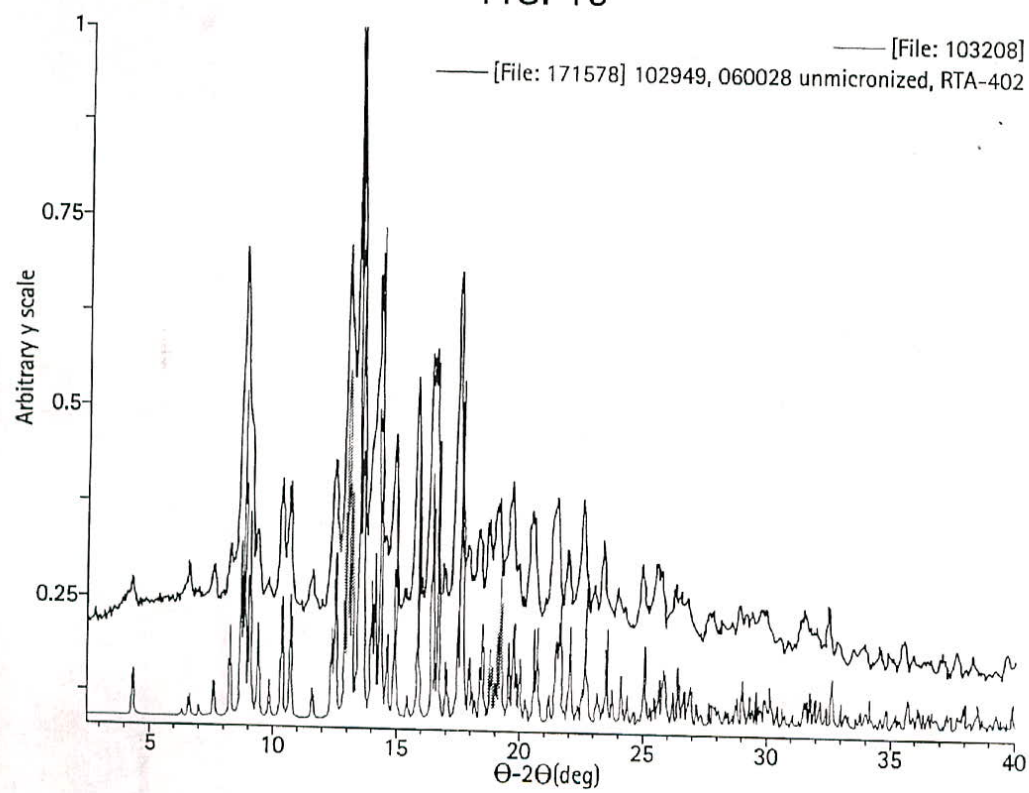


FIG. 19



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FIG. 20

Form A vs Amorphous in Cynomolgus Monkeys
Following a 4.1 mg/kg oral dose

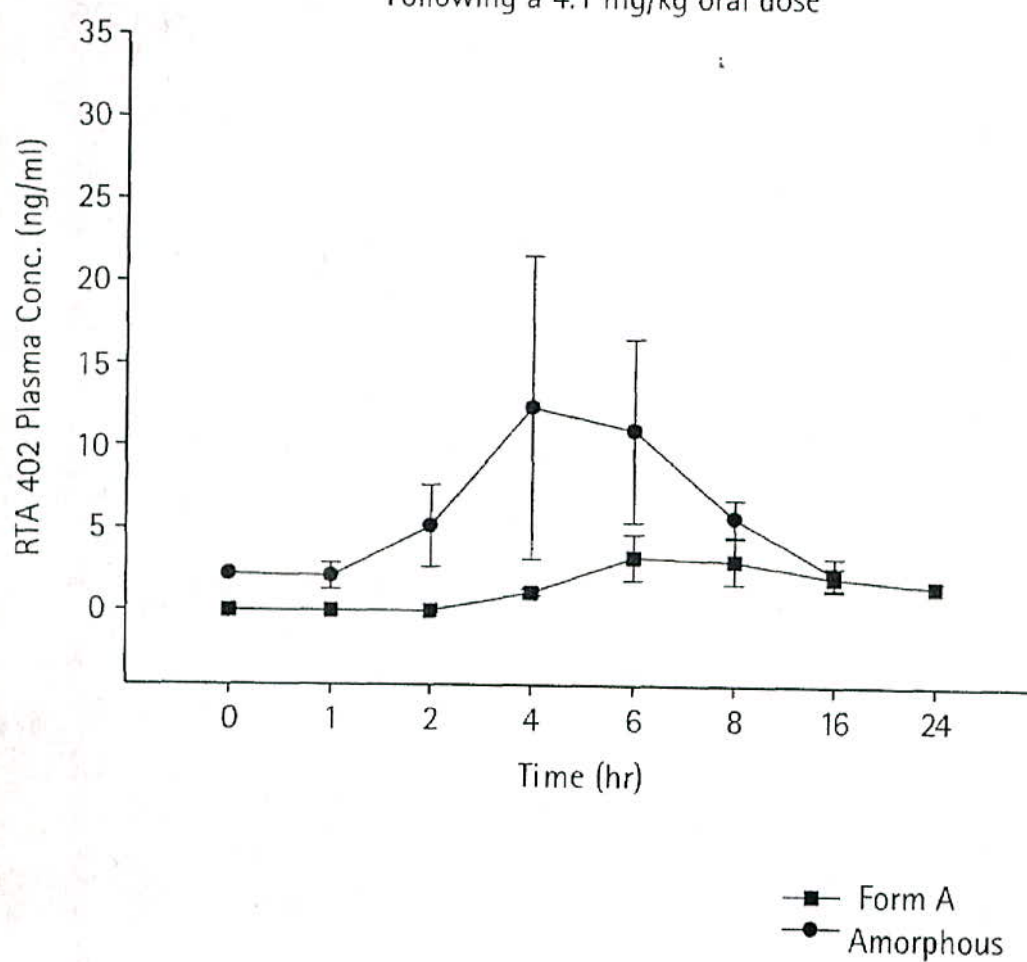
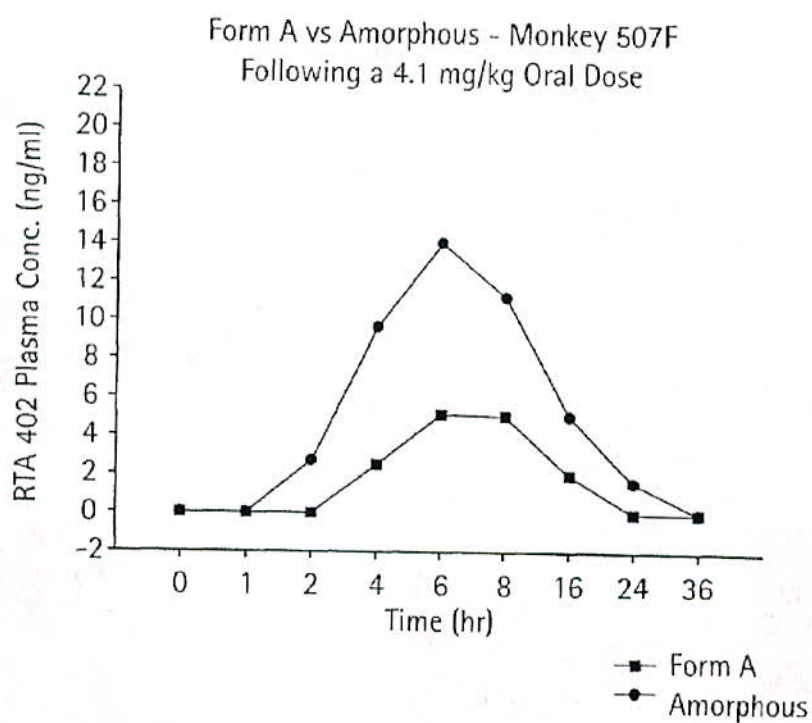
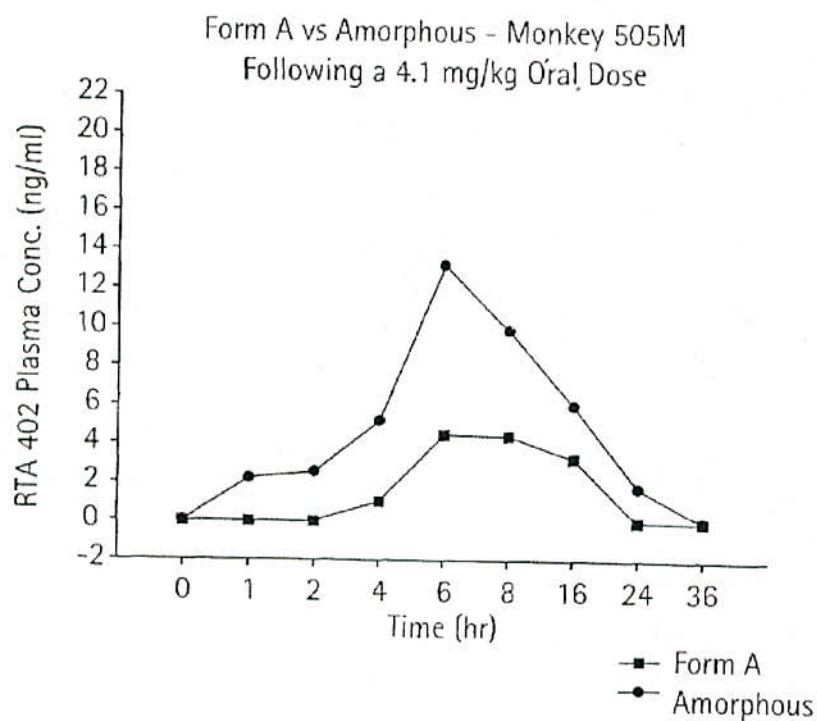
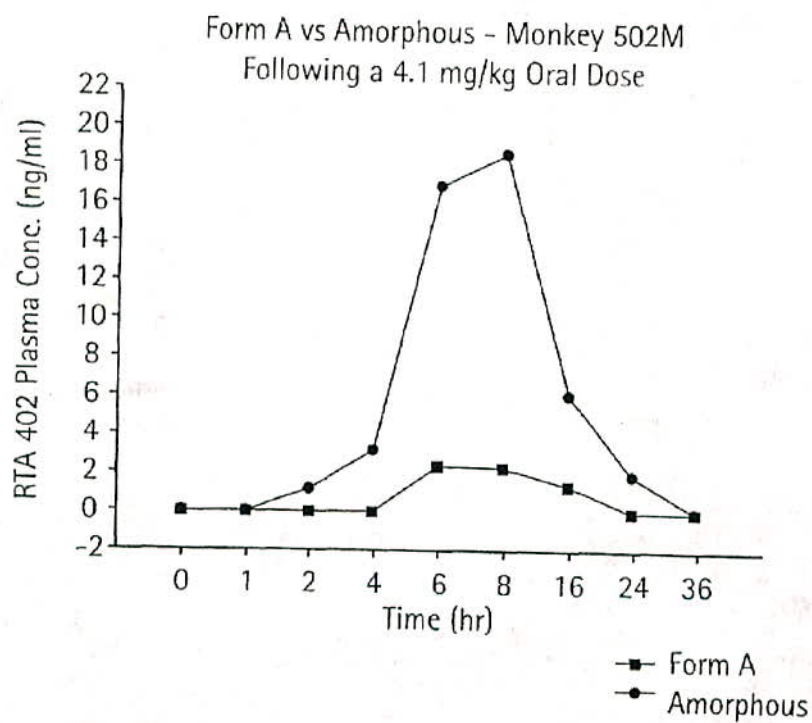
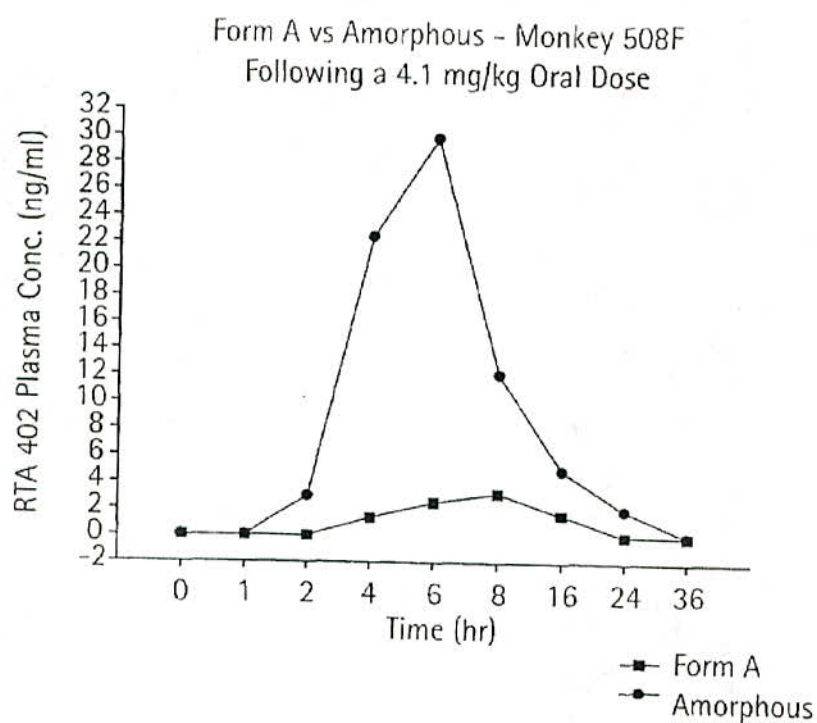


FIG. 21



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FIG. 22



23

FIG. 23

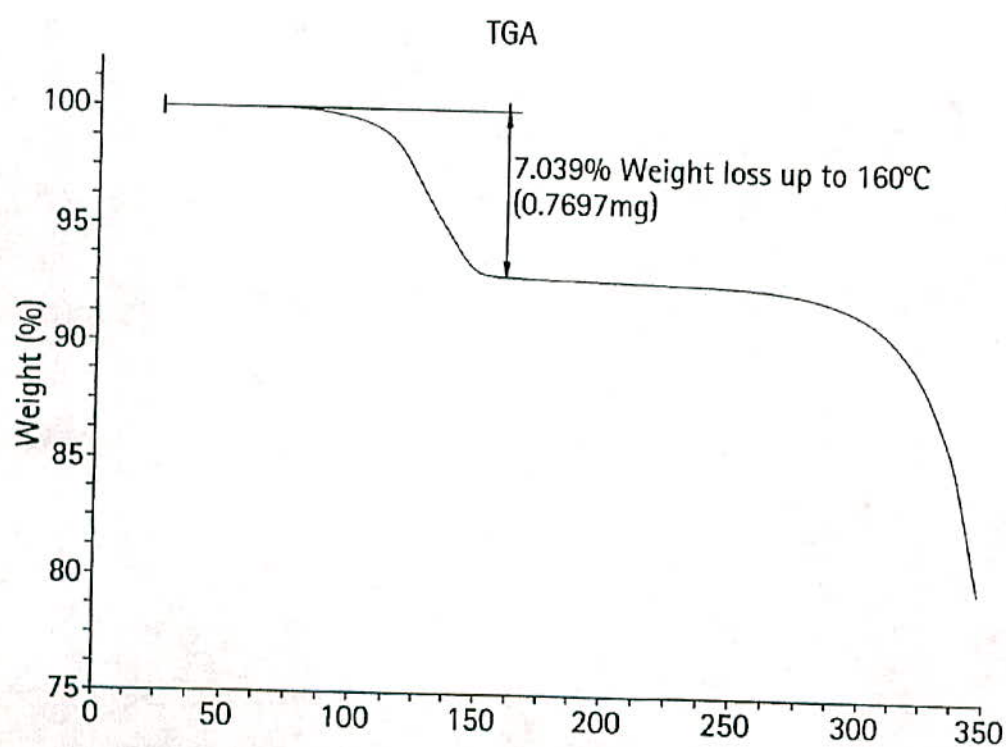
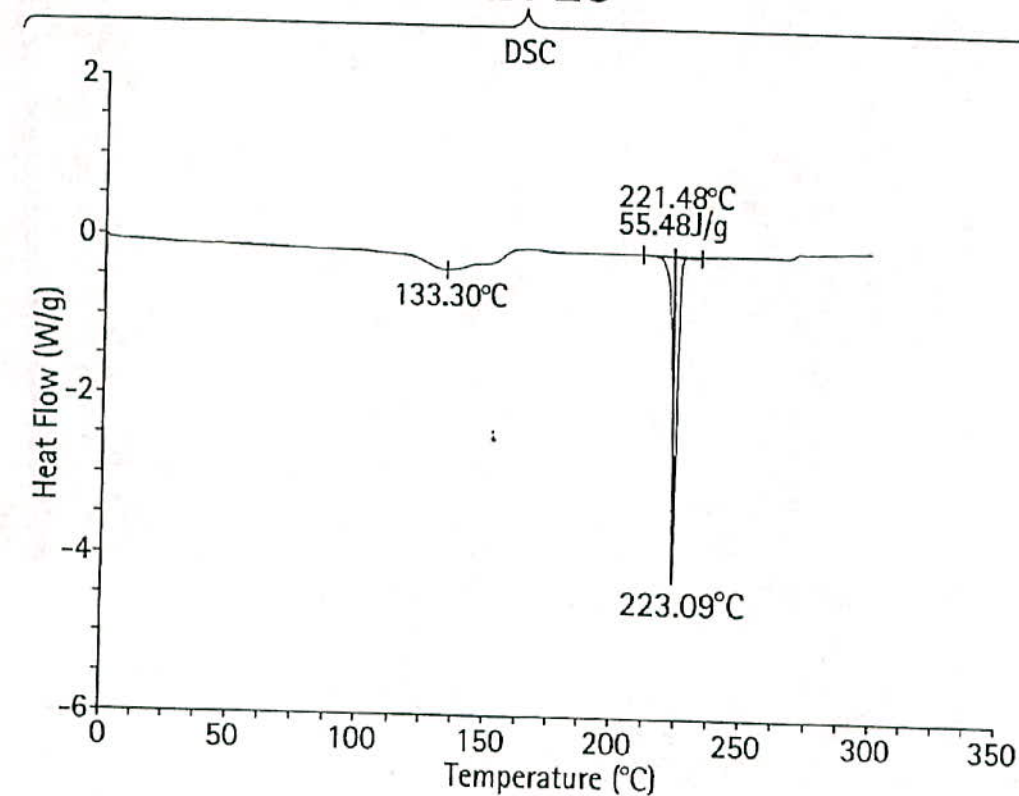
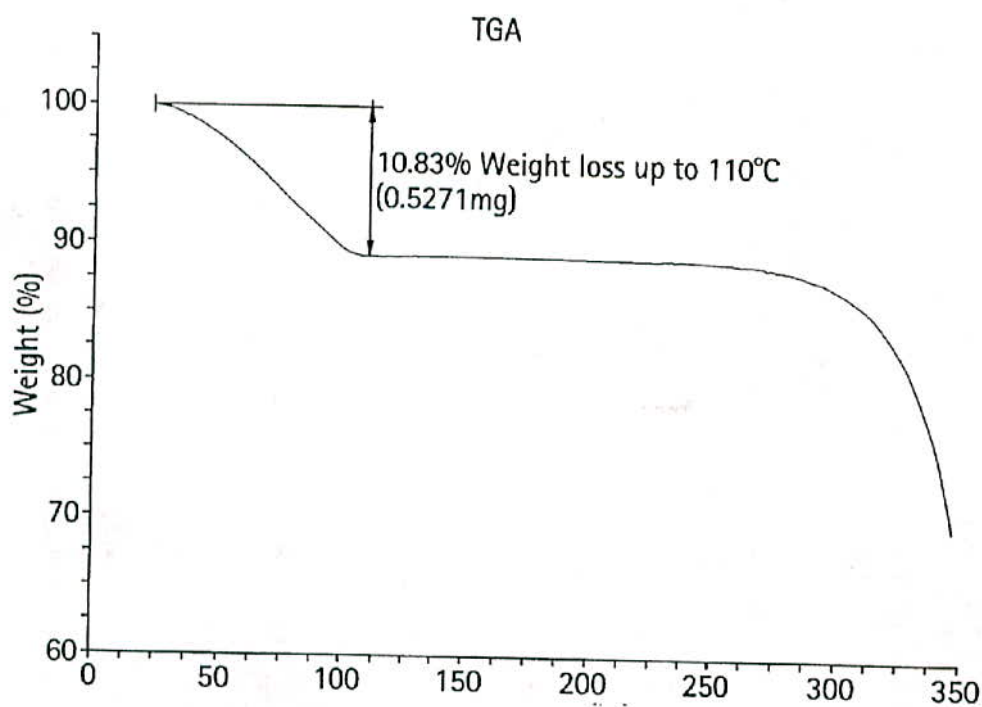
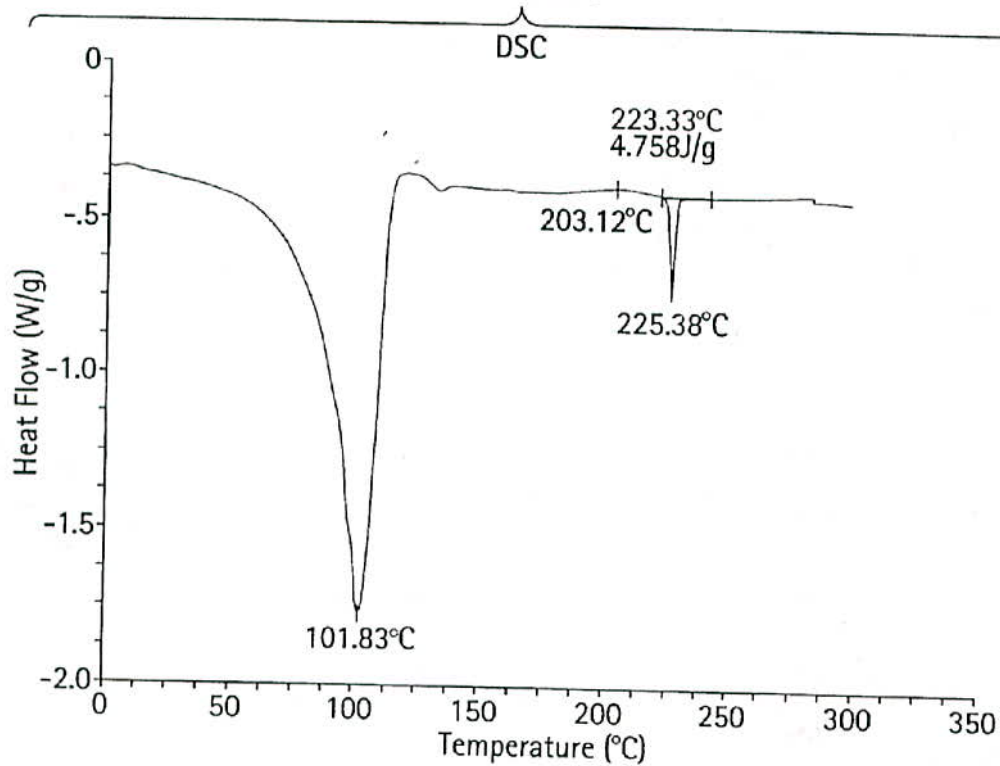


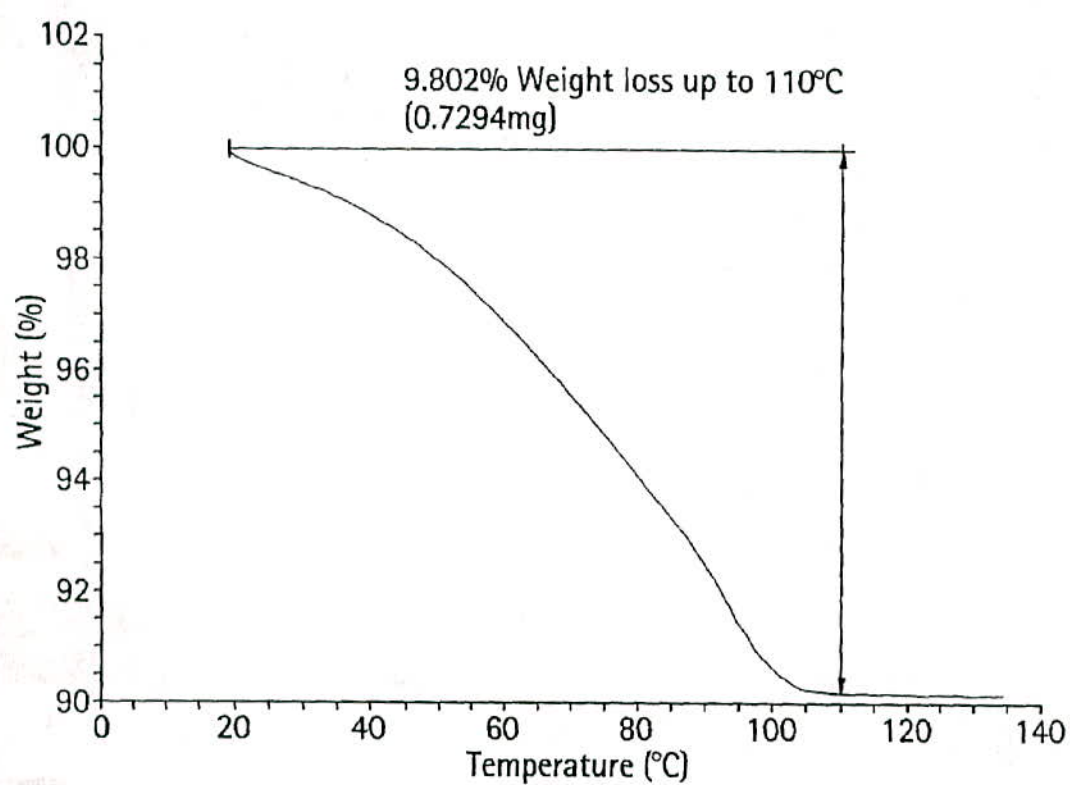
FIG. 24



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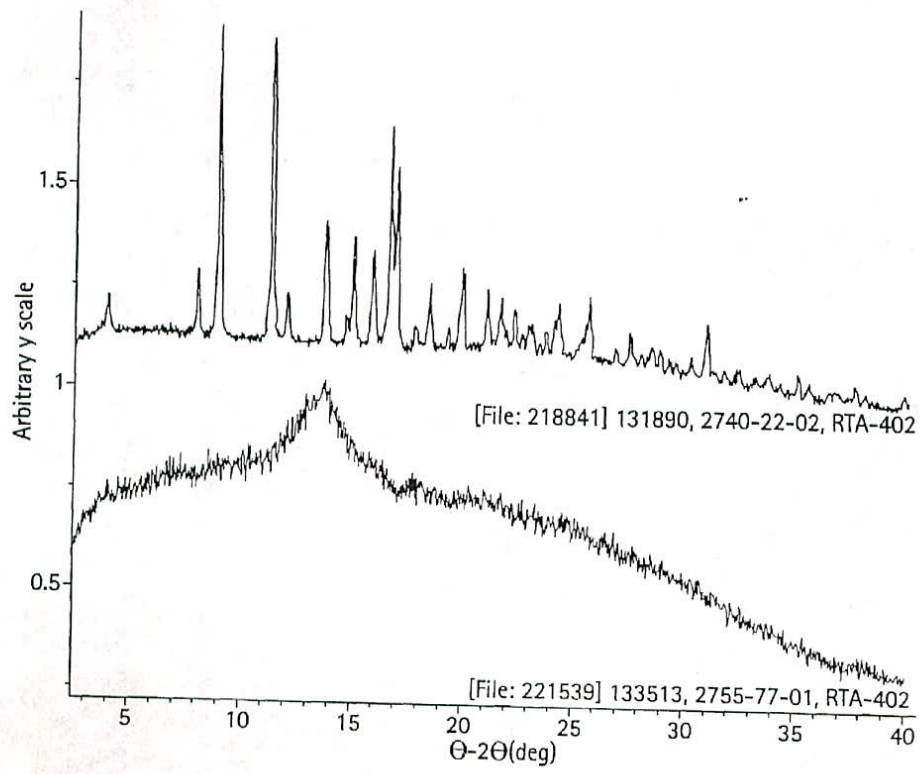
FIG. 25

TGA



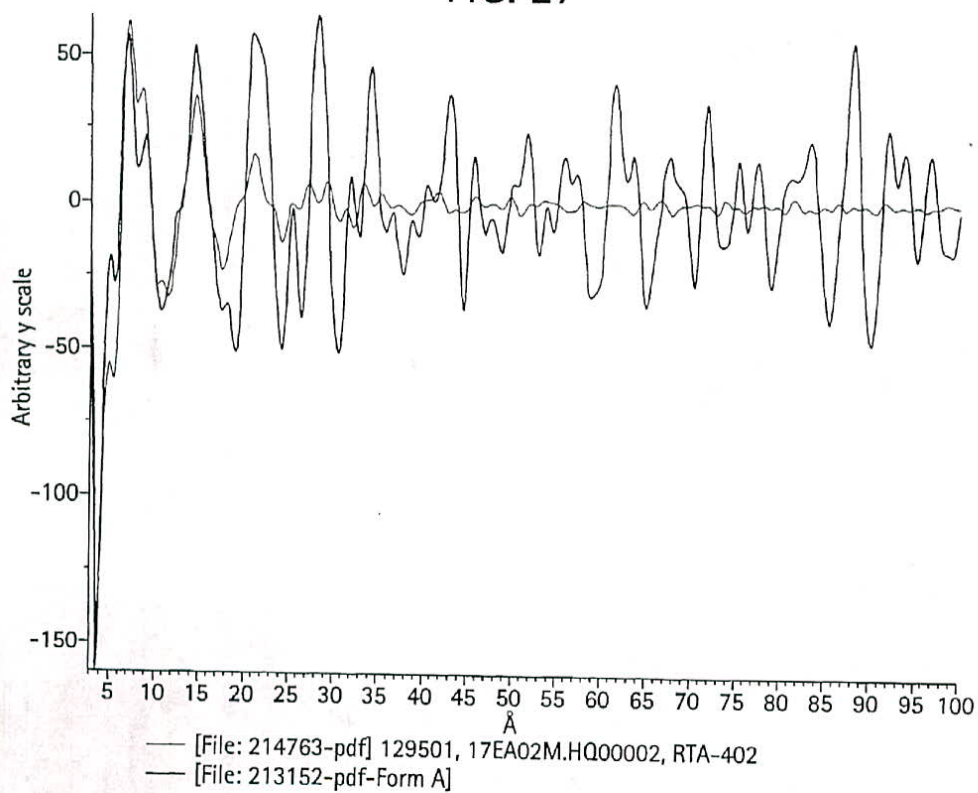
37

FIG. 26



34

FIG. 27



3r

FIG. 28

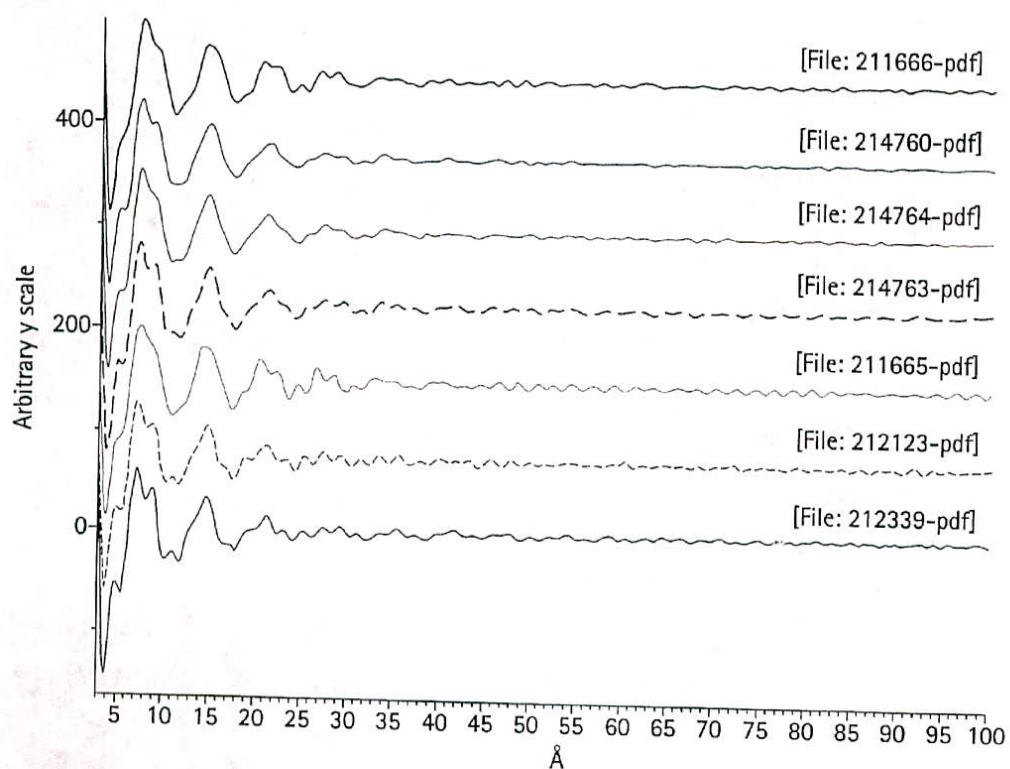


FIG. 29

$P4_32_12$ (#96)

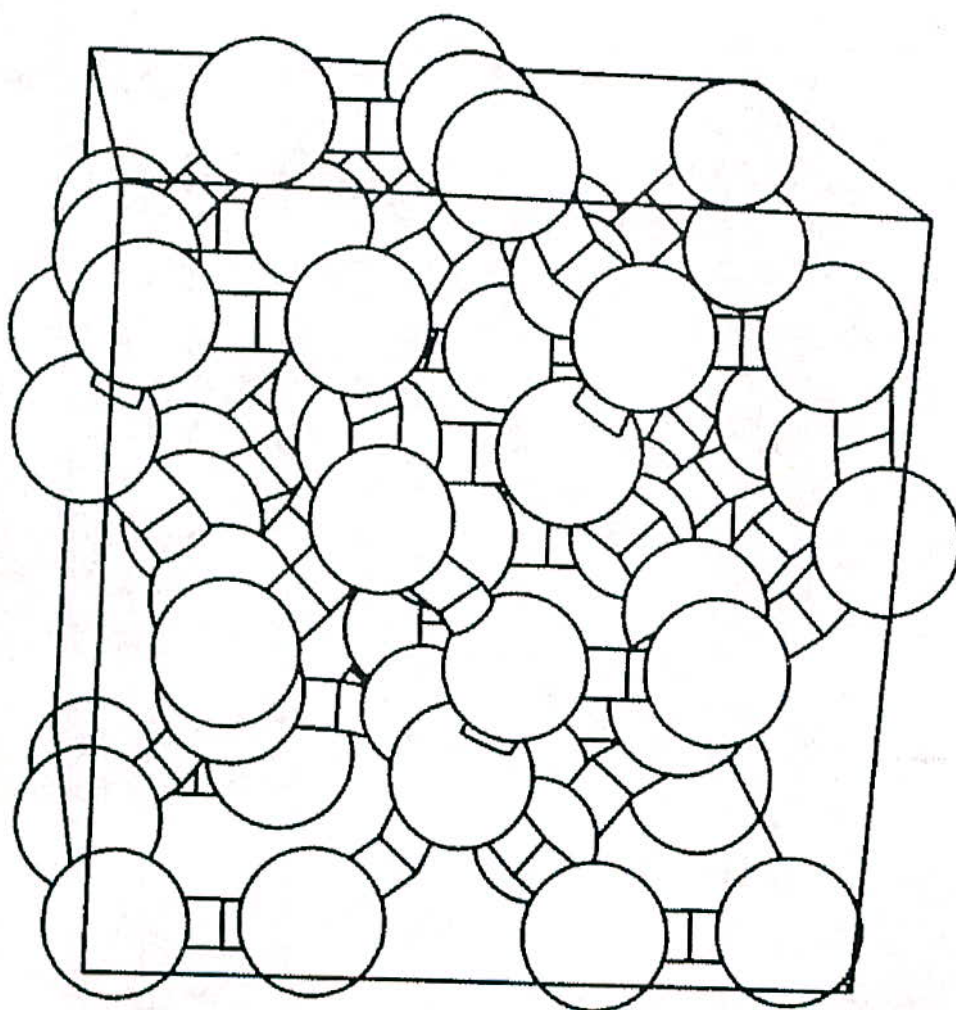
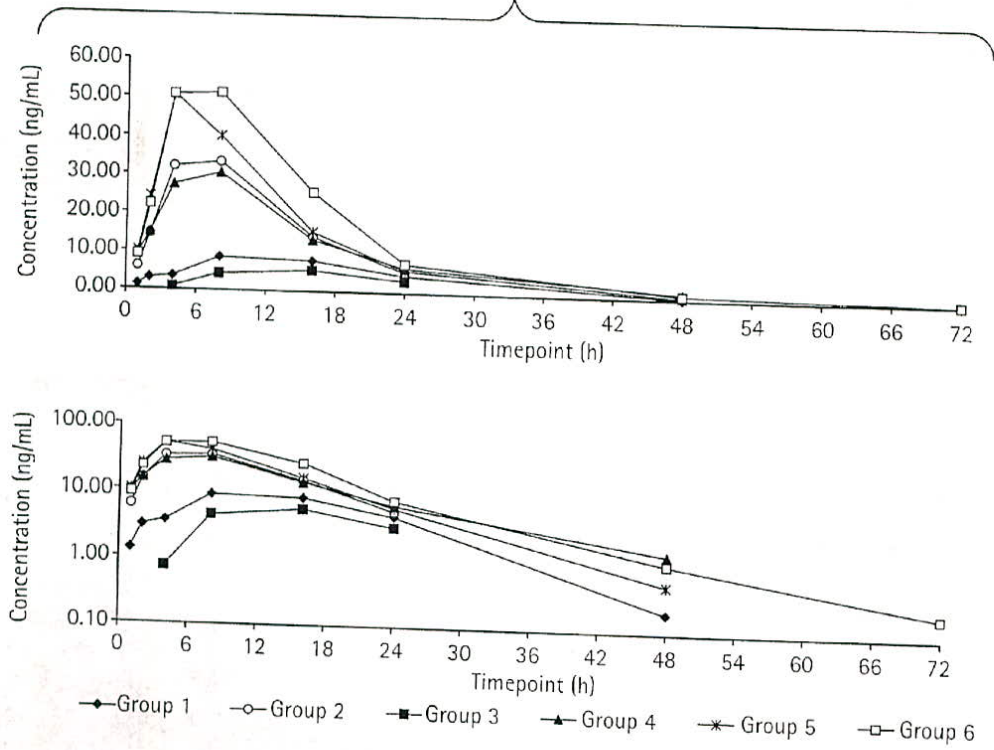


FIG. 30



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NOVEL FORMS OF CDDO METHYL ESTER

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims priority from U.S. Provisional Application 60/955,939, filed Aug. 15, 2007, incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Triterpenoids are biosynthesized in plants by the cyclization of squalene. Although candidates for medicinal use, these naturally occurring molecules display relatively weak biological activity. Accordingly, chemists have sought to synthesize analogues of enhanced potency (Honda et al., 1997 & 1998).

[0003] Several synthetic analogs are reported to suppress the de novo formation of iNOS and COX-2 in macrophages that have been stimulated by IFN- γ or LPS (Suh et al., 1998; Honda et al., 2002). Another synthetic triterpenoid, 2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oate (CDDO), exhibits anti-inflammatory and anti-proliferative activity (Honda et al., 1998 & 2000).

[0004] Studying the methyl ester of CDDO, which is methyl 2-cyano-3,12-dioxoleana-1,9(11)-dien-28-oate (CDDO methyl ester), Bore et al. (2002) determined a crystal structure. In that form, which is hydrated, water coordinates interactions that engender a particular crystal packing and structure.

SUMMARY OF THE INVENTION

[0005] In one embodiment of the present invention, a non-hydrous crystalline form of CDDO methyl ester is provided. The non-hydrous crystal form preferably has a space group of $P4_3 2_1 2$ with unit cell dimensions of $a=14.2 \text{ \AA}$, $b=14.2 \text{ \AA}$, and $c=81.6 \text{ \AA}$. The invention also contemplates a pharmaceutical composition in solid dosage form, comprising (i) a therapeutically effective amount of a non-hydrous crystalline form of CDDO methyl ester with (ii) an edible carrier.

[0006] Additionally, the present invention is embodied in a glassy solid form of CDDO methyl ester, having an x-ray powder diffraction pattern with a halo peak at approximately $13.5^\circ 2\theta$, as shown in FIG. 2C, and a glass transition temperature (T_g). In particular embodiments, the T_g can range from about 120°C . to about 135°C . In other embodiments, the T_g ranges from about 125°C . to about 130°C . The glassy solid form of CDDO-methyl ester can have a PDF spectrum with similar peaks to FIG. 28 from about $5 \text{ \AA}$ to about $20 \text{ \AA}$.

[0007] Furthermore, the invention provides a pharmaceutical composition in solid dosage form, comprising (i) a therapeutically effective amount of a glassy solid form of CDDO methyl ester with (ii) an edible carrier. In this regard, the invention contemplates a methodology for treating a cancer patient, comprising: administering such a pharmaceutical composition to a cancer patient. The invention also contemplates administering the glassy form of CDDO-methyl ester in combination with another anti-cancer drug. For example, the anti-cancer drug may be gemcitabine and the cancer may be pancreatic cancer. The invention encompasses as well a methodology for the treatment of diseases or disorders that involve acute or chronic oxidative stress and inflammation, particularly those characterized in part by overexpression of inducible nitric oxide synthase (iNOS) or inducible cyclooxygenase (COX-2).

[0008] In addition, the invention is drawn to a dimethanol solvate form of CDDO-methyl ester, having an x-ray powder diffraction pattern with characteristic peaks as shown in Table 18 and a DSC pattern as shown in FIG. 24. Pursuant to the invention, the dimethanol solvate form may be used as an intermediate for the production of a glassy solid form of CDDO methyl ester. A method for the production of the glassy solid form of CDDO-methyl ester, via the dimethanol solvate form, comprises preparing a dimethanol solvate form of CDDO-methyl ester and drying the dimethanol solvate form.

[0009] In accordance with another embodiment, the invention is drawn to a method of growing a crystal of CDDO methyl ester dimethanolate, comprising preparing a solution of purified CDDO methyl ester in warm anhydrous methanol, adding the warm solution to a vessel of chilled methanol, and filtering the resulting crystals.

[0010] In accordance with another embodiment, the invention is drawn to a pharmaceutical composition comprising (i) a therapeutically effective amount of CDDO-methyl ester and (ii) an excipient that is a glass former, such that the composition has a T_g .

[0011] The excipient may be selected, for instance, from the group consisting of (A) a carbohydrate, carbohydrate derivative, or carbohydrate polymer, (B) a synthetic organic polymer, (C) an organic acid salt, (D) a protein, polypeptide, or peptide, and (E) a high molecular weight polysaccharide. Illustrative of the class of synthetic organic polymer excipients are a hydroxypropyl methyl cellulose, such as hydroxypropyl methyl cellulose phthalate ester, a poly[1-(2-oxo-1-pyrrolidinyl)ethylene or copolymer thereof, such as PVP/VA, and a methacrylic acid copolymer, such as methacrylic acid-ethyl acrylate:copolymer (1:1).

[0012] Another exemplary excipient in this regard is copovidone, which is 1-vinyl-2-pyrrolidone-vinyl acetate copolymer (3:2).

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 depicts the chemical structure of CDDO methyl ester.

[0014] FIG. 2 presents the XRPD pattern of Form A (top) and Form B (bottom). From top to bottom: unmiconized Form A; micronized Form A; and Form B.

[0015] FIG. 3 shows the DSC and TG curve of CDDO methyl ester (Form A).

[0016] FIG. 4 shows the hot stage analysis of Form A—unmicronized.

[0017] FIG. 5 shows the dynamic vapor sorption isotherm of Form A—unmicronized.

[0018] FIG. 6 shows the SEM images of Form A—unmicronized.

[0019] FIG. 7 shows Form A before (top) and after (bottom) stress at 195°C .

[0020] FIG. 8 shows the NMR spectrum of Form A—unmicronized.

[0021] FIG. 9 shows the MDSC curve of Form B CDDO methyl ester.

[0022] FIG. 10 shows Form B CDDO methyl ester before (top) and after (bottom) thermal stress at 200°C /ambient RH for 60 minutes.

[0023] FIG. 11 shows the NMR spectrum of Form B CDDO methyl ester.

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[0024] FIG. 12 shows an ORTEP drawing of a single Form A molecule with labeling. Atoms are represented by 50% probability anisotropic thermal ellipsoids.

[0025] FIG. 13 shows an ORTEP drawing of the contents of the asymmetric unit of Form A crystals. Atoms are represented by 50% probability anisotropic thermal ellipsoids.

[0026] FIG. 14 shows a packing diagram of Form A crystals viewed down the crystallographic a axis.

[0027] FIG. 15 shows a packing diagram of Form A crystals viewed down the crystallographic b axis.

[0028] FIG. 16 shows a packing diagram of Form A crystals viewed down the crystallographic c axis.

[0029] FIG. 17 shows the calculated X-ray powder pattern of Form A.

[0030] FIG. 18 shows the experimental XRPD of Form A.

[0031] FIG. 19 presents a comparison of the calculated and experimental XRPD patterns for Form A CDDO methyl ester.

[0032] FIG. 20 shows a representative plot of the area under the curve for Form A and Form B, following a 4.1 mg/kg oral administration to cynomolgus monkeys. Each datum point represents the mean plasma concentration of CDDO methyl ester in 8 animals. Error bars represent the standard deviation within the sampled population.

[0033] FIG. 21 shows a comparison of plasma concentration of Form B CDDO methyl ester versus Form A in Animal #505M (top panel) and animal #507F (bottom panel).

[0034] FIG. 22 presents a comparison of plasma concentration of Form B CDDO methyl ester versus Form A between Animal #508F (top panel) and animal #502M (bottom panel).

[0035] FIG. 23 depicts thermograms of CDDO methyl ester hemibenzenesolvate.

[0036] FIG. 24 shows thermograms of CDDO-methyl ester dimethanol solvate.

[0037] FIG. 25 depicts TGIR data relating to CDDO methyl ester dimethanol solvate.

[0038] FIG. 26 presents XRPD patterns of CDDO methyl ester dimethanol solvate, before (top) and after (bottom) TGIR analysis (up to 140° C.).

[0039] FIG. 27 is an overlay representation of PDF data for Form A vs. Form B. Local order is similar from about 5 Å to about 20 Å.

[0040] FIG. 28 is an overlay representation of X-ray amorphous patterns for different preparations of Form B, showing substantial uniformity among the preparations.

[0041] FIG. 29 is a schematic representation of space group $P4_32_12$ (#96).

[0042] FIG. 30 shows the mean blood concentrations of CDDO-methyl ester following single oral administrations of CDDO-methyl ester capsules to male Cynomolgus monkeys (Phases 2 and 3).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0043] As noted, the study of triterpenoids as suppressors of iNOS activity, and specifically in the inhibition of NO production, has demonstrated the high potency of CDDO and CDDO methyl ester ($IC_{50} < 1$ nM level). See Honda et al. (2000). These studies focused on solubilized CDDO methyl ester, providing little characterization of a solid of CDDO methyl ester. The work of Bore et al. (2002) elucidated a structure, the first published for a triterpenoid, of a single, solvated crystalline form of CDDO methyl ester.

[0044] To realize the therapeutic potential of CDDO methyl ester, depicted in FIG. 1 (chemical structure) and in

FIG. 12 (ORTEP drawing), the present inventors investigated other forms of the compound that possessed properties, such as greater aqueous solubility and chemical stability, that are advantageous to development of a medicinal product with suitable pharmacokinetics. Consequently, the inventors discovered two forms of CDDO methyl ester, distinct from the crystal form elucidated by Bore et al. (2002), that have such properties and, hence, are candidates for drug development in their own right.

[0045] Inventive "Form A" of CDDO methyl ester is unsolvated (non-hydrous) and is characterized by a distinctive crystal structure, with a space group of $P4_32_12$ (no. 96), shown in FIG. 29, unit cell dimensions of $a=14.2$ Å, $b=14.2$ Å and $c=81.6$ Å, and by a packing structure, depicted in FIGS. 14-16, whereby three molecules are packed in helical fashion down the crystallographic b axis. Table 10 below enumerates additional crystal data for Form A, along with crystallographic data-collection parameters.

[0046] The other "Form B" of the invention is in a single phase but lacks such a defined crystal structure. Rather, Form B is typified by an x-ray powder diffraction (XRPD) spectrum differing from that of Form A (see FIG. 2, inter alia). Moreover, Form B displays a bioavailability that is surprisingly better than that of Form A (see Example 7).

[0047] Methodology for the synthesis of CDDO methyl ester has been published. See U.S. Pat. No. 6,326,507, Honda et al. (1998), and Honda et al. (2000). The inventors have discovered that both Form A and Form B of CDDO methyl ester are readily prepared from a variety of solutions of the compound, illustrated by those detailed in Table 3-5, infra. In particular, Form B can be prepared by fast evaporation or slow evaporation in MTBE, THF, toluene, or ethyl acetate. By the same token, Form A can be prepared via fast evaporation, slow evaporation, or slow cooling of a CDDO methyl ester solution in ethanol or methanol. Preparations of CDDO methyl ester in acetone can produce either Form A, using fast evaporation, or Form B, using slow evaporation. Additional preparation methods are described below, including the tables provided there.

[0048] Since it does not have a defined crystal structure, Form B likewise lacks distinct XRPD peaks, such as those that typify Form A, and instead is characterized by a general "halo" XRPD pattern. In particular, the non-crystalline Form B falls into the category of "x-ray amorphous" solids because its XRPD pattern exhibits three or fewer primary diffraction halos (see FIG. 10, for instance). Within this category, Form B is a "glassy" material: As shown by the PDF, the nearest neighbor atom-atom interactions match that observed for crystalline Form A, but the notion of an average unit cell does not apply because there is no long-range order manifested.

[0049] Unlike Form A, therefore, samples of Form B show no long-range molecular correlation, i.e., above roughly 20 Å (see FIG. 27). Moreover, thermal analysis of Form B samples reveals a glass transition temperature (T_g). In contrast, a disordered nanocrystalline material, does not display a T_g but instead only a melting temperature (T_m), above which crystalline structure becomes a liquid.

[0050] The present description also characterizes a CDDO-methyl ester dimethanol solvate form that can be used to prepare form B (see Example 9). Also characterized here is a CDDO-methyl ester hemibenzenate form (see Example 8).

[0051] Although micronization of other crystalline materials has been found to affect XRPD spectra, XRPD analysis of micronized Form A results in a spectrum similar to unmicon-

ized Form A. See FIG. 2 for a side-by-side comparison of unmiconized Form A, micronized Form A, and Form B CDDO methyl ester.

[0052] Various means of characterization can be used together to distinguish Form A and Form B CDDO methyl ester from each other and from other forms of CDDO methyl ester. Illustrative of the techniques suitable for this purpose are solid state Nuclear Magnetic Resonance (NMR), X-ray powder diffraction, X-ray crystallography, Differential Scanning Calorimetry (DSC), dynamic vapor sorption/desorption (DVS), Karl Fischer analysis (KF), hot stage microscopy, modulated differential screening calorimetry, FT-IR, and Raman spectroscopy.

[0053] In particular, analysis of the XRPD and DSC data can distinguish Form A, Form B, and hemibenzenate forms of CDDO-methyl ester.

[0054] The properties of the inventive CDDO methyl ester forms are both distinctive, as mentioned above, and conducive to their use as medicinal agents. For example, the bioavailability of Form B and Form A CDDO methyl ester varied in monkeys when the monkeys received equivalent dosages of the two forms orally, in gelatin capsules. See Example 7. In addition, the stability of the newly identified CDDO-methyl ester forms will be useful in the production of pharmaceutical compositions.

In similar manner to distinguishing Form A and Form B CDDO methyl ester from each other and from other forms of CDDO methyl ester, CDDO methyl ester dispersions that retain "x-ray amorphous" character, as described in greater detail below, can be distinguished from dispersions containing crystalline Form A CDDO methyl ester by a variety of techniques, including XRPD and DSC analysis. Thus, dispersions containing Form A crystalline CDDO methyl ester typically display discrete peaks characteristic of the pure Form A CDDO methyl ester, particularly those that occur at approximately 13.35 and 8.78 ($^{\circ}2\theta$) (for example, see Table 17, *infra*).

[0055] The properties of a CDDO methyl ester polymer excipient dispersion of the invention are both distinctive and conducive to their use as medical agents. For example, the bioavailability of selected CDDO methyl ester dispersions, formulated with additional inactive additives, varied in monkeys when the monkeys received equivalent dosages of the dispersions in gelatin capsules. See Example 7, *infra*, study phases 2 and 3. In several instances, formulations containing CDDO methyl polymer excipient dispersions produced surprising further enhancements in bioavailability, even relative to formulations produced from pure Form B CDDO methyl ester.

[0056] The presence of multiple forms, including polymorphs, in pharmaceutical solids has been previously described, for instance, by Cui (2007). The crystalline and amorphous forms of a compound may exhibit different physical and chemical characteristics. For instance, amorphous forms may have higher solubility relative to the crystalline form. Every compound is unique in this regard, however, and the degree to which an amorphous material will differ from the crystalline state must be investigated on a case-by-case basis and cannot be predicted *a priori*. In addition, some amorphous materials are prone to re-crystallization.

[0057] In the present context, variability in data collection can arise due to a myriad of factors. Accordingly, this description uses the terms "about" or "approximately" to indicate variations in data used to describe the CDDO-methyl ester

forms. For example, a melting temperature may vary based on instrumentation or conditions. Regarding the precision of the measurement, the USP <891> states that "In the case of melting, both an "onset" and a "peak" temperature can be determined objectively and reproducibly, often to within a few tenths of a degree." Practical experience indicates this is not true for measuring the T_g of a material. The T_g will depend on many factors: how the sample was prepared, the thermal history of the sample (relaxation), residual solvent that may or may not volatilize prior to T_g , the instrument, sample preparation (sample mass, particle size, packing, diluents), the parameters used to measure T_g (particularly scan rate), the parameters used to determine the location of the T_g (onset temperature, mid-point temperature, inflection point temperature, or offset temperature), whether a relaxation endotherm is present at T_g , and other factors. Some factors will decrease T_g (plasticization due to residual water/solvent), while others will increase T_g (faster scan rate, relaxation) and may do so by as much as 10-15 $^{\circ}$ C. The change in heat capacity at T_g (ΔC_p) can be important, as reported by Zhou et al., *J Pharmaceutical Sciences* 91: 1863-72 (2002).

[0058] The present description speaks of different patterns in terms of their "characteristic" peaks. The assemblage or group of such peaks is unique to a given polymorphic form, within the uncertainty attributable to individual instruments and to experimental conditions, respectively.

[0059] For each of the crystalline forms, a group of five characteristic peaks is listed in Tables 17-19, below. Typical variation can be $\pm 0.1^{\circ} 2\theta$, but peak position can vary up to $\pm 0.2^{\circ} 2\theta$ or more in some experiments.

TABLE 17

Form A Peak Position ($^{\circ}2\theta$)
13.35
8.78
17.4
12.94
14.18

TABLE 18

Dimethanolate Peak Position ($^{\circ}2\theta$)
8.87
11.26
16.63
16.9
13.72

TABLE 19

Hemibenzenate Peak Position ($^{\circ}2\theta$)
14.17
9.25
16.32
14.62
17.11

[0060] The XRPD pattern of the glassy material (Form B) shows a broad halo peak at approximately 13.5 $^{\circ} 2\theta$, which

appears to be characteristic of Form B. Other halos are not as well-defined, and the shape/position of this pattern may change as a function of the instrument and experimental conditions. Variation in the position of this broad peak will be larger than that of the characteristic peaks of the respectively crystalline forms. In particular, variability of up to $\pm 1^\circ 20'$ for the broad peak of Form B can be expected in certain instruments.

[0061] The XRPD pattern of glassy materials produced as CDDO methyl ester excipient dispersions also show a broad halo peak, typically centered at approximately $13.5^\circ 2\theta$. These materials likewise display a T_g by modulated Differential Scanning Calorimetry (mDSC). Similar to pure Form B CDDO methyl ester samples, the shape and position of the XRPD pattern for an excipient dispersion may change as a function of the instrument used, the experimental conditions, and the specific excipient employed to produce the dispersions.

[0062] The present invention further relates to the use of Form A, Form B, and glassy, XRPD-amorphous excipient dispersions of CDDO methyl ester, respectively, for treating diseases associated with inflammation, including a cancerous condition and various pathologies affecting the central nervous system. Pursuant to the invention, treatment of these diseases comprises administering to a subject in need thereof an effective amount of the novel CDDO methyl ester forms enumerated here. These compounds have utility for ameliorating or preventing inflammation involved in the etiology of cancer, Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), rheumatoid arthritis (RA) and other autoimmune diseases, inflammatory bowel disease, and other pathological conditions tied to excessive production of either nitric oxide or prostaglandins.

[0063] As previously noted, the aberrant or excessive expression of either cyclooxygenase-2 (COX-2) or inducible nitric oxide synthase (iNOS) has been implicated in the pathogenesis of many disease processes, including carcinogenesis in the colon. Several synthetic analogs of triterpenoids, including CDDO methyl ester, have been reported to suppress iNOS expression. Related studies have shown triterpenoid suppression of both iNOS and COX-2 expression in macrophages that have been stimulated by IFN- γ or LPS (Suh et al., 1998; Honda et al., 2002). Therefore, treatments administering CDDO methyl ester forms are expected to affect iNOS and COX-2 suppression.

[0064] Overexpression of the gene for COX-2 is an early and central event in colon carcinogenesis (Prescott and White, 1996; Dubois et al., 1996). Mice with defects in the APC (adenomatous polyposis coli) gene develop large numbers of intestinal polyps at an early age, and marked elevations in COX-2 enzyme levels have been found in these polyps. These animal findings correlate with the finding of elevated levels of COX-2 mRNA and protein in many human primary colon cancers and colon cancer cell lines (Prescott and White, 1996), and it is believed that this elevation in COX-2 leads to a suppression of apoptosis, which would ordinarily lead to death of pre-neoplastic cells (Tsujii and DuBois, 1996). The functional relevance of COX-2 to intestinal tumorigenesis has been demonstrated by knockout of the COX-2 gene (Oshima et al., 1996). Mice bearing this knockout were mated with polyp-forming mice bearing lesions in the APC gene; the COX-2 knockout caused a dramatic diminution in the number of polyps in the offspring. Furthermore,

treatment of experimental animals with either selective COX-2 inhibitors or non-selective COX-1/COX-2 inhibitors has been reported to be a potent approach to chemoprevention of intestinal cancer (Mamett, 1992; Oshima et al., 1996; Boolbol et al., 1996; Reddy et al., 1996; Sheng et al., 1997). As for the role of iNOS in carcinogenesis, it is clear that NO is a potent mutagen (Tamiir and Tannebaum, 1996), and that nitric oxide can also activate COX-2 (Salvemini et al., 1993, 1994). There also is a marked increase in iNOS in rat colon tumors induced by the carcinogen, azoxymethane (Takahashi et al., 1997). Similarly, overexpression of iNOS in human tumors has been reported as a negative prognostic factor (e.g., Ekemekioglu et al., 2006).

[0065] Inflammatory signaling pathways and other disease-associated signaling pathways, such as are induced by angiotensin II, frequently stimulate excessive production of reactive oxygen or nitrogen species (RONS), including superoxide, hydrogen peroxide, nitric oxide and peroxynitrite. CDDO methyl ester has been shown to be a potent inducer of antioxidant activity and a potent inhibitor of inflammatory processes in many different cell types (Dinkova-Kostova et al., 2005; Liby et al., 2006; Ahmad et al., 2006; Shishodia et al., 2006). Severe, acute inflammation due to a variety of causes including infection, trauma, burns, and chemical exposure can be life threatening and may cause liver failure, kidney failure, respiratory failure, or heart failure. Chronic inflammation and the associated oxidative stress contribute to the pathology of many important diseases including autoimmune diseases (e.g., rheumatoid arthritis, lupus, psoriasis, and multiple sclerosis), cardiovascular diseases (e.g., atherosclerosis and heart failure), diabetes (type I and type II), respiratory diseases (e.g., chronic obstructive pulmonary disease and asthma), chronic kidney disease, renal failure, liver failure, and pain syndromes (e.g., neuropathic pain, fibromyalgia, and migraine). In addition, triterpenoids have been shown to inhibit the replication of HIV-1 in macrophages (Vazquez et al., 2005) and so may be useful in the treatment of viral diseases, particularly those in which significant morbidity is caused by organ or tissue inflammation (e.g., viral hepatitis, influenza, herpes simplex).

[0066] MS is known to be an inflammatory condition of the central nervous system (Williams, Ulvestad and Hickey, 1994; Merrill and Benevise, 1996; Genain and Nauser, 1997). Inflammatory, oxidative, or immune mechanisms may be involved in the pathogenesis of MS, AD, PD, and ALS (Bagasra et al., 1995; Griffin et al., 1995; McGeer and McGeer, 1995; Good et al., 1996; Simonian and Coyle, 1996; Kaltschmidt et al., 1997). Both reactive astrocytes and activated microglia have been implicated in causation of NDD/NID; there has been a particular emphasis on microglia as cells that synthesize both NO and prostaglandins as products of the respective enzymes, iNOS and COX-2. De novo formation of these enzymes may be driven by inflammatory cytokines such as interferon-gamma or interleukin-1. In turn, excessive production of NO may lead to inflammatory cascades and/or oxidative damage in cells and tissues of many organs, including neurons and oligodendrocytes of the nervous system, with consequent manifestations in AD and MS, and possibly PD and ALS (Coyle and Puttfarcken, 1993; Goodwin et al., 1995; Beal, 1996; Good et al., 1996; Merrill and Benvenist, 1996; Simonian and Coyle, 1996; Vodovotz et al., 1996). Epidemiologic data indicate that chronic use of NSAID's, which block synthesis of prostaglandins from arachidonate, markedly lowers the risk for development of

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AD (McGeer et al., 1996; Stewart et al., 1997). Accordingly, Form A and Form B of CDDO methyl ester, as agents that block formation of NO and prostaglandins, should be useful in therapeutic approaches to treating or preventing NDD.

[0067] As described above, in a variety of preclinical studies CDDO-Me has demonstrated an ability to inhibit the expression of COX-2 and iNOS, enzymes associated with both inflammation and carcinogenesis. CDDO-Me also was shown to inhibit the activation of nuclear factor-kappa B (NF- κ B) and Signal Transducer and Activator of Transcription 3 (STAT3), transcription factors associated with inflammation, tumor progression, and tumor resistance to therapy. Initial studies evidenced that CDDO-Me inhibited the growth of many cancer cell lines; the average IC₅₀ value for CDDO-Me in the NCI-60 tumor cell line panel was approximately 35 nM. In vivo studies confirmed that CDDO-Me effectively inhibited the growth of tumors formed by human tumor cell lines implanted in rodents or syngeneic cancer cell lines implanted in rodents (Table 16). Doses used in these studies were generally in the range of 10 to 100 mg/kg/day, depending on the species, strain, and method of administration.

[0068] Studies detailed below provide human data that reflect the beneficial effect of CDDO-methyl ester on patients suffering from a cancerous condition. See Example 10.

[0069] In light of the foregoing, the present invention encompasses stable, controlled release dosage forms containing a CDDO methyl ester form. A dosage form of the invention can be for once-per-day administration, for delayed release, or for pulsatile release, thereby to optimize therapy by matching pharmacokinetic performance with pharmacodynamic requirements.

[0070] Any of Form B, Form A, and formulations containing excipient dispersions of CDDO methyl ester may be administered orally. The active compound may be coated in a material to protect the compound from the action of acids and other natural conditions which may inactivate the compound. Other modes of administration, such as topical, subcutaneous, intravenous, and intraperitoneal are also part of the current invention.

[0071] To administer the therapeutic compound, it may be necessary to coat the compound with or to co-administer the compound with a material to prevent its inactivation. Thus, either Form B or Form A CDDO methyl ester may be administered to a subject in an appropriate carrier, such as liposomes, or in a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes. See, e.g., Strejan et al., *J. Neuroimmunol* 7: 27 (1984).

[0072] The therapeutic compound can be administered orally, with inert diluents, additives, or an edible carrier, to form a pharmaceutical composition. To this end, the therapeutic compound of the invention, with other ingredients, may be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, Form A or Form B may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Similarly, an excipient dispersion of the present invention may be presented in a variety of dosage form types, including those described here for Form A or Form B. The percentage of the therapeutic compound in the compositions and preparations

may be varied, in accordance with conventional practice, to effect a suitable dosage of the active agent.

[0073] Additionally, the present invention relates to a pharmaceutical composition comprising an effective amount of Form B CDDO methyl ester or Form A, in association with one or more non-toxic, pharmaceutically acceptable carriers and/or diluents and, if desired, other active ingredients. As noted above, the active compound can be produced as a homogeneous excipient dispersion, starting from either Form B or Form A. Such a CDDO methyl ester excipient dispersion is a solid solution and can be viewed as a homogeneous dispersion at the molecular level. Such dispersions can be advantageously formulated together with other pharmaceutically acceptable additives, to stabilize the active compound and, in some instances, to provide further improvements in bioavailability.

[0074] In formulating CDDO methyl ester as an excipient dispersion, the choice of an excipient for the dispersion is guided by the criteria that the excipient be both a good "glass former" and pharmaceutically acceptable. More generally, the excipient should form a stable, homogeneous glassy matrix, which stabilizes the dispersion by affording a T_g that is above typical, ambient temperature storage conditions. An additional criterion in this regard is that the excipient used for the dispersion should be chemically compatible with other additives, such as binders, fillers, lubricants, glidants, and the like, which may be employed in the final formulation to confer desired functional properties.

[0075] In satisfaction of these criteria, an excipient can be selected, pursuant to the invention, from a number of compounds characterized by suitably high T_g values, such as (A) carbohydrates, carbohydrate derivatives, and carbohydrate polymers, (B) synthetic organic polymers, (C) organic acid salts, (D) proteins, polypeptides, and peptides, and (E) high molecular weight polysaccharides such as heparin, which is a sulfated polysaccharide, and hyaluronic acid, a mucopolysaccharide.

[0076] Illustrative of class (A) are: cellulose derivatives, such as hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC), and ethyl cellulose; polysaccharides, such as raffinose, maltotriose, stachyose, dextrans (including maltodextrins and cyclodextrins, inter alia), dextrans, and soluble starch; alditols, such as mannitol, xylitol, and sorbitol; and disaccharides, such as lactose, trehalose, maltose, and sucrose. A preferred excipient from this class is hydroxypropyl methyl cellulose phthalic ester (HPMC-P).

[0077] Class (B) is exemplified by poly[1-(2-oxo-1-pyrrolidinyl)ethylene, a/k/a povidone or polyvinylpyrrolidone (PVP) and related co-polymers, such as PVP/VA, of varying molecular weights. Also included in this class is the methacrylic acid family of copolymers, such as methacrylic acid copolymer Type C (USP/NF).

[0078] Class (C) is illustrated by salts, such as sodium, potassium, calcium and magnesium salts, of lactic acid, ascorbic acid, maleic acid, oxalic acid, malonic acid, malic acid, succinic acid, citric acid, gluconic acid, and glutamic acid, respectively. Thus, representative salts in this regard are sodium citrate, sodium lactate, sodium maleate, magnesium gluconate, and sodium ascorbate.

[0079] Exemplary class (D) excipients are: human serum albumin; a polyamino acid, e.g., polyalanine, polyarginine, polyglycine, and polyglutamic acid; casein; collagen; gelatin and purified gelatin proteins; and certain pharmacologically active compounds, such as insulin.

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[0080] Excipients can alter some of the physical characteristics of the pharmaceutical formulations, as noted. For instance, dispersion within the various polymeric excipients may lead to a reduction in the observed T_g of the formulation. Normally, T_g is an additive property based on proportions of materials involved. Accordingly, when utilizing polymers with T_g values that are less than that of the amorphous Form B, there is an expectation of a reduction in observed T_g for the dispersions (mixtures). Additionally, moisture or traces of residual organic solvent often are present, which tends to reduce T_g as well.

For purposes of generating a solid CDDO-Me dispersion, the optimal choice of excipient typically must be determined empirically. For example, attempts to produce glassy, XRPD-amorphous dispersions using the polyethylene glycol (PEG) family of excipients, such as PEG 6000, produced mixtures containing characteristic peaks associated with the presence of Form A CDDO methyl ester. Similar results were obtained using Vitamin E-TPGS, an excipient produced by esterifying d-alpha-tocopheryl acid succinate with polyethylene glycol 1000, as well as using ethylene oxide-propylene oxide copolymers such as Pluronic®. As the examples below illustrate, certain polymeric excipients used to form dispersions with CDDO-Me, pursuant to the invention, display surprising improvements in oral bioavailability relative to the pure Form B drug substance.

[0081] Methods can vary for preparing homogenous, glassy, X-ray amorphous dispersions of CDDO-Me with pharmaceutically acceptable excipients, and the examples presented here utilize spray drying to generate such dispersions. Other methods of manufacture may be used to produce dispersions of the invention with equivalent properties and utility. See Repka et al. 2002, and references cited therein. Such other methods include but are not limited to solvent evaporation and extrusion, such as hot melt extrusion.

[0082] In addition to an excipient, other additives may be included to aid in stability of the active ingredient, to adjust the pH (i.e., a buffering agent), to improve dispersibility, to aid in providing uniformity of delivery, and to achieve other characteristics desired for a pharmaceutical formulation.

[0083] The administered quantity of the compound or composition of the present invention will vary, depending on the patient and the mode of administration, and can be any effective amount.

[0084] A given treatment regimen for the administration of a composition of the present invention can be developed by way of normal and routine pre-clinical and clinical testing, the details of which are a function of the therapeutic indication, among other factors. The quantity of the active agent administered may vary over a wide range, thereby to provide, in a unit dosage, a pharmacologically effective amount based upon the body weight of the patient per day, to achieve the desired effect. The desired dosage may also vary according to the condition being treated. For example, treatment of acute cancer may require a significantly higher dose than treatment of an inflammatory condition such as arthritis.

[0085] In particular, a composition of the present invention is presented as a unit dose and taken preferably from 1 to 3 times daily, most preferably once daily to achieve the desired effect.

[0086] In addition, a composition of the current invention may be taken every two days, every three days, every four days, every five days, every six days, or once a week.

[0087] The compositions of the current invention also may be administered alone or in combination with other drugs based on the particular needs of a patient. In particular, the compositions of the current invention may be administered

with anti-cancer agents as part of a treatment regimen. For example, CDDO-methyl ester may be administered with gemcitabine, or other agents, during the treatment of a cancer, such as pancreatic cancer.

[0088] In general, pharmaceutical compositions of the invention are prepared using conventional materials and techniques, such as mixing, blending and the like. Moreover, a medicament containing Form A or Form B also can contain other components, including but not limited to suitable adjuvants, carriers, excipients, and stabilizers, etc. A therapeutic formulation of the invention is preferably a solid but, in principle, could be a liquid, such as a suspension or emulsion.

[0089] Pursuant to the invention, the oral maintenance dose typically is between about 0.1 mg and about 1000 mg, preferably given once daily. The dosage may be varied or keyed to a subjects weight. Typical dosages may be from about 0.01 mg/kg to 100 mg/kg, with the preferred unit dosage forms including tablets and capsules.

[0090] The following examples are illustrative only and are not intended to limit the present invention. The materials and methods employed in the examples are outlined below:

[0091] a. Materials

[0092] Solvents and other reagents were purchased from commercial suppliers and were either HPLC or ACS grade.

[0093] b. Experimental Methods

[0094] i. Approximate Solubility—Solvent Addition Method

[0095] A weighed sample was treated with aliquots of the test solvent at room temperature. Complete dissolution of the test material was determined by visual inspection. Solubility was estimated based on the total solvent used to provide complete dissolution. The actual solubility may be greater than the value calculated because of the use of solvent aliquots that were too large or due to a slow rate of dissolution. The solubility is expressed as "less than" if dissolution did not occur during the experiment. If complete dissolution was achieved as a result of only one aliquot addition, the solubility is expressed as "greater than."

[0096] ii. Polymorph Screen

[0097] Both thermodynamic and kinetic crystallization techniques were employed. These techniques are described in more detail below. Once solid samples were harvested from crystallization attempts, they were either examined under a microscope for morphology or observed with the naked eye. Any crystalline shape was noted, but sometimes the solid exhibited unknown morphology, due to small particle size. Solid samples were then analyzed by XRPD, and the patterns were compared to each other to identify new crystalline or non-crystalline forms.

[0098] (i) Cold Precipitation (CP)

[0099] Solutions were prepared in various solvents at elevated temperature. The solutions were then filtered through a 0.2-µm nylon or PTFE filter into an antisolvent at sub-room temperature. The presence or absence of solids was noted. If there were no solids present, or if the amount of solids was judged too small for XRPD analysis, the vial was placed in a freezer. The resulting solids were isolated by filtration and dried prior to analysis.

[0100] (ii) Fast Evaporation (FE)

[0101] Solutions were prepared in various solvents and sonicated between aliquot additions to assist in dissolution. Once a mixture reached complete dissolution, as judged by visual observation, the solution was filtered through a 0.2-µm nylon filter. The filtered solution was allowed to evaporate at room temperature in an uncapped vial. The solids that formed were isolated and analyzed.

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[0102] (iii) Freeze Dry (FD)

[0103] 1,4-dioxane solutions were prepared, filtered through a 0.2- μ m nylon filter, and frozen using dry ice. The frozen sample was lyophilized using an FTSystems Flexi-Dry. The lyophilization temperature was not controlled.

[0104] (iv) Micronization

[0105] Micronization of materials can be accomplished in fluid energy mills and can reduce particle size to 1 to 20 microns. Further description of these processes can be found in PERRY'S CHEMICAL ENGINEERS' HANDBOOK, 7th ed. (McGraw Hill, 1998).

[0106] (v) Grinding

[0107] A solid sample was placed into a stainless steel milling rotor with a small metal ball. Some samples had a small amount of water added (wet grinding). The sample was then ground at 30 Hz on a Retesh type MM220 mixermill for approximately 20 minutes. The resulting solids were isolated and analyzed.

[0108] (vi) Cryogrinding

[0109] A solid sample was placed into a stainless steel grinding jar with a grinding rod. The sample was then ground at 15 Hz on a SPEX Certiprep model 6750 cryomill for a set amount of time. The grinding jar was submerged in a bath of liquid nitrogen during the experiment. The solids were isolated and analyzed.

[0110] (vii) Melt/Quench

[0111] A solid sample was placed on a glass microscope slide and leveled. The slide was then placed on a hot plate at a set temperature until the solid melted. Upon melting, the slide was removed from the hot plate and placed on a cold counter top to cool quickly. The resulting solids were dried under nitrogen and analyzed.

[0112] (viii) Rotary Evaporation

[0113] Solutions were prepared in various solvents and filtered through a 0.2- μ m nylon filter. The sample was placed on the rotary evaporator and removed when dry. The resulting solids were isolated and analyzed.

[0114] (ix) Slow Evaporation (SE)

[0115] Solutions were prepared in various solvents and sonicated between aliquot additions to assist in dissolution. Once a mixture reached complete dissolution, as judged by visual observation, the solution was filtered through a 0.2- μ m nylon filter. The filtered solution was allowed to evaporate at room temperature or under nitrogen in a vial covered with aluminum foil perforated with pinholes. The solids thus formed were isolated and analyzed.

[0116] (x) Slow Cool (SC)

[0117] Saturated solutions were prepared in various solvents at approximately 60° C. and filtered through a 0.2- μ m nylon filter into an open vial while still warm. The vial was covered and allowed to cool slowly to room temperature. The presence or absence of solids was noted. If there were no solids present, or if the amount of solids was judged too small for XRPD analysis, the vial was placed in a refrigerator. Again, the presence or absence of solids was noted and, if there were none, the vial was placed in a freezer. Solids that formed were isolated by filtration and allowed to dry prior to analysis.

[0118] (xi) Slurry Experiments

[0119] Solutions were prepared by adding enough solids to a given solvent so that excess solids were present. The mixture was then agitated in a sealed vial at room temperature. After either 7 or 10 days, the solids were isolated by vacuum filtration and analyzed.

(xii) Stress Experiments

[0120] Solids were stressed under different temperature and/or relative humidity (RH) environments for a measured

time period. Specific RH values were achieved by placing the samples inside sealed chambers containing saturated salt solutions or inside ESPEC temperature and humidity cabinets. The salt solutions were selected and prepared following an ASTM standard procedure. Samples were analyzed by XRPD immediately after removal from the stress environment.

[0121] iii. Single Crystal Structure Determination

[0122] (i) Sample Preparation

[0123] A saturated solution of CDDO methyl ester was prepared in methanol at ~60° C. and filtered through a 0.2- μ m filter into an open vial while still warm. The vial was covered and allowed to cool slowly to room temperature. The presence of pyramidal tablets was observed after 1 day.

[0124] (ii) Data Collection

[0125] A colorless plate of CDDO-Me ($C_{32}H_{43}NO_4$) having approximate dimensions on two sides of 0.01 $\times$ 0.01 mm, was mounted on a glass fiber in random orientation. Preliminary examination and data collection were performed with Mo K_{α} radiation ($\lambda=0.71073$ Å) on a Nonius KappaCCD diffractometer equipped with a graphite crystal, incident beam monochromator. Refinements were performed on a LINUX PC using SHELX97 (Sheldrick, 1997) [1].

[0126] Cell constants and an orientation matrix for data collection were obtained from least-squares refinement using the setting angles of 46742 reflections in the range $2^{\circ}<\theta<22^{\circ}$. The refined mosaicity from DENZO/SCALEPACK [2] was 0.32° indicating good crystal quality. The space group was determined by the program XPREP [3]. From the systematic presence of the following conditions: $h00$ $h=2n$, $00l$ $l=4n$, and from subsequent least-squares refinement, the space group was determined to be $P4_32_12$ (no. 96).

[0127] The data were collected to a maximum 2θ value of 44.43°, at a temperature of 150 $\pm$ 1 K.

[0128] (iii) Data Reduction

[0129] Frames were integrated with DENZO-SMN [2]. A total of 46742 reflections were collected, of which 9168 were unique. Lorentz and polarization corrections were applied to the data. The linear absorption coefficient is 0.074 mm⁻¹ for Mo K_{α} radiation. An empirical absorption correction using SCALEPACK [2] was applied. Transmission coefficients ranged from 0.9995 to 0.9999. Intensities of equivalent reflections were averaged. The agreement factor for the averaging was 9.3% based on intensity.

[0130] (iv) Structure Solution and Refinement

[0131] The structure was solved by direct methods using SHELXS97 [1]. The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were included in the refinement but restrained to ride on the atom to which they are bonded. The structure was refined in full-matrix least-squares by minimizing the function:

$$\sum w(|F_o|^2 - |F_c|^2)^2$$

The weight w is defined as $1/[\sigma^2(F_o^2) + (0.0176P)^2 + (0.0000P)]$, where $P = (F_o^2 + 2F_c^2)/3$.

[0132] Scattering factors were taken from the "International Tables for Crystallography" [5]. Of the 9168 reflections used in the refinements, only the reflections with $F_o^2 > 2\sigma(F_o^2)$ were used in calculating R . A total of 5421 reflections were used in the calculation. The final cycle of refinement included 1024 variable parameters and converged (largest parameter shift was <0.01 times its estimated standard deviation) with unweighted and weighted agreement factors of:

$$R = \sum |F_o - F_c| / \sum F_o = 0.051$$

$$R_w = \sqrt{(\sum w(F_o^2 - F_c^2)^2) / \sum w(F_o^2)^2} = 0.085$$

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The standard deviation of an observation of unit weight was 1.05. The highest peak in the final difference Fourier had a height of 0.22 e/Å³. The minimum negative peak had a height of -0.25 e/Å³.

[0133] (v) Calculated X-Ray Powder Diffraction (XRPD) Pattern

[0134] A calculated XRPD pattern was generated for Cu radiation using PowderCell 2.3 [6] and the atomic coordinates, space group, and unit cell parameters from the single crystal data.

[0135] (vi) ORTEP and Packing Diagrams

[0136] The ORTEP diagram was prepared using ORTEP III [7][9]. Atoms are represented by 50% probability anisotropic thermal ellipsoids. Packing diagrams were prepared using CAMERON [8] modeling software. Additional figures and BFDH morphology predictions were generated using Mercury 1.4.1 [4].

[0137] c. Instrumental Techniques

[0138] i. Differential Scanning Calorimetry (DSC)

[0139] Analyses were carried out on a TA Instruments differential scanning calorimeter 2920 or Q1000. The instrument was calibrated using indium as the reference material. The sample was placed into a standard aluminum DSC pan with a crimped lid configuration, and the weight accurately recorded. The sample cell was equilibrated at 25° C. and heated under a nitrogen purge at a rate of 10° C./min, up to a final temperature of 250° C.

[0140] ii. Dynamic Vapor Sorption/Desorption (DVS)

[0141] Moisture sorption/desorption data were collected on a VTI SGA-100 Vapor Sorption Analyzer. Sorption and desorption data were collected over a range of 5% to 95% relative humidity (RH) at 10% RH intervals under a nitrogen purge. Samples were not dried prior to analysis. Equilibrium criteria used for analysis were less than 0.010% weight change in 5 minutes, with a maximum equilibration time of 3 hours if the weight criterion was not met. Data were not corrected for the initial moisture content of the samples. Sodium chloride and polyvinylpyrrolidone were used as calibration standards.

[0142] iii. Karl Fischer (KF)

[0143] Coulometric Karl Fischer (KF) analysis for water determination was performed using a Mettler Toledo DL39 Karl Fischer titrator. Approximately 24-32 mg of sample was placed in the KF titration vessel containing Hydranal—Coulomat AD. The sample was then titrated by means of a generator electrode which produces iodine by electrochemical oxidation:

$2\text{I}^- \rightarrow \text{I}_2 + 2\text{e}^-$. Three Replicates were Obtained to Ensure Reproducibility.

[0144] iv. Hot Stage Microscopy

[0145] Hot stage microscopy was performed using a Linkam hot stage (model FTIR 600) mounted on a Leica DM LP microscope. Samples were observed using a 20× objective (obj.) with cross polarizers (CP) and lambda (λ) compensator. Samples were placed on a coverslip. A second coverslip was then placed over the sample. Each sample was visually observed as the stage was heated. Images were captured using a SPOT Insight™ color digital camera with SPOT Software v. 4.5.9. The hot stage was calibrated using USP melting point standards.

[0146] v. Modulated Differential Scanning Calorimetry (MDSC)

[0147] Modulated differential scanning calorimetry data were obtained on a TA Instruments differential scanning calorimeter equipped with a refrigerated cooling system (RCS). The sample was placed into an aluminum DSC pan, and the weight accurately recorded. The pan was covered with a lid

and crimped. MDSC data were obtained using a modulation amplitude of $\pm 0.8^\circ\text{C}$. and a 60 second period with an underlying heating rate of 2° C./min from -25 to 250° C. The temperature and the heat capacity were calibrated using indium metal and sapphire as the calibration standards, respectively. The reported glass transition temperature is obtained from the inflection of the step change in the reversible heat flow versus temperature curve.

[0148] vi. Nuclear Magnetic Resonance (NMR)

[0149] The solution phase ¹H NMR spectra were collected by Spectra Data Services, Inc. Acquisition parameters are printed on each spectrum. Spectra were referenced to internal tetramethylsilane at 0.0 ppm.

[0150] vii. Optical Microscopy

[0151] Observations made by optical microscopy were collected on a Wolfe polarizing optical microscope at a magnification of 4×. Crossed polarizers (CP) were used to observe birefringence in the samples.

[0152] viii. Scanning Electron Microscopy (SEM)

[0153] Scanning electron microscopy (SEM) was performed using a FEI Quanta 200 scanning electron microscope. Under high vacuum mode, a solid state backscatter (Etd) detector was used. Beam voltage was 5.0 kV. Samples were sputter coated using a Cressington 108 auto Sputter Coater at -20 mA and -0.13 mbar (Ar) with Au/Pd for 75 seconds. Samples were prepared for analysis by placing a small amount on carbon double-stick tape fixed to an aluminum sample mount. The instrument was calibrated for magnification using NIST standards. Data was collected using xTm (v. 2.01), build number 1564 and analyzed using XT Docu (v. 3.2). Magnifications reported on the SEM images were calculated upon the initial data acquisition. The scale bar reported in the lower portion of each image is accurate upon resizing the images and should be utilized when making size determinations.

[0154] ix. Thermogravimetry (TG)

[0155] Analyses were carried out on a TA Instruments 2950 thermogravimetric analyzer. The calibration standards were nickel and Alumel™. Each sample was placed in an aluminum sample pan and inserted into the TG furnace. Samples were first equilibrated at 25° C., then heated under a stream of nitrogen at a heating rate of 10° C./min, up to a final temperature of 350° C. unless specified otherwise.

[0156] x. X-Ray Powder Diffraction (XRPD)

[0157] (i) Inel XRG-3000

[0158] X-ray powder diffraction analyses were also performed on an Inel XRG-3000 diffractometer, equipped with a curved position-sensitive detector with a 2θ range of 120°. Real time data was collected using Cu Kα radiation at a resolution of 0.03° 2θ. The tube voltage and amperage were set to 40 kV and 30 mA, respectively. Patterns are displayed from 2.5 to 40° 2θ to facilitate direct pattern comparisons. Samples were prepared for analysis by packing them into thin-walled glass capillaries. Each capillary was mounted onto a goniometer head that is motorized to permit spinning of the capillary during data acquisition. Instrument calibration was performed daily using a silicon reference standard.

[0159] d. Additional Calculation Techniques

[0160] i. PDF

[0161] One technique used in the computational analysis of x-ray amorphous data is the Pair Distribution Function (PDF). As the name suggests, the PDF is made up from a linear sum of all the coherent atom-atom interactions within the material. Defective (disordered) materials will exhibit the same atom-atom interactions as the crystalline phase, but over a reduced length scale. Therefore, such materials can be compared to parent crystalline materials by examining the peaks

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in the PDF over the first few nanometers. Comparisons between spectra in the range of 0 Å to about 5 Å are difficult due to artifacts in this region.

[0162] On the formation of glassy material, the PDF peaks will exhibit some movement from the crystalline peak positions as the molecules relax their positions. This is similar to a thermal expansion/contraction event where, although the PDF peaks move slightly, the relative peak intensities should still be recognizable as being related to the original crystalline material. As the material enters the thermodynamic amorphous state, some of the point group symmetry relationships will be lost giving a PDF of reduced complexity. There also will be some peak movement. The glassy/amorphous materials will exhibit PDFs that will rapidly fall to zero over 2 to 3 nearest neighbor (NN) distances.

[0163] Measurement conditions are used to minimize the background in the x-ray patterns and algorithms are used to calculate the PDF from measured x-ray data. The PDFs were calculated using PatternMatch v2.2.1, using the entire range of measured data for all samples.

Example 1

Solubility Estimates

[0164] Approximate solubilities were determined in various solvents at room temperature, with the results as provided in Table 2. CDDO methyl ester exhibits high solubility in the majority of the organic solvents used. The solubility in water appears to be less than 0.1 mg/mL.

Example 2

Polymorph Screen Results

[0165] Approximately 50 polymorph screen experiments were performed. Form A was observed from approximately 50% of the samples. The formation of Form A was not limited to a particular crystallization condition and was prepared from a variety of different experiments and solvents. Form B material was prepared from lyophilization, melt/quench, and several evaporation experiments.

[0166] Polymorph screen samples are listed alphabetically in Tables 3-5 by the solvent used. Representative XRPD patterns of Form A and Form B materials are compared in FIG. 2. Characterization data of the forms are summarized in the examples below.

Example 3

Characterization of CDDO-Methyl Ester—Form A (Unmicronized)

[0167] Form A is unsolvated (Table 6). The single crystal structure of Form A was determined based on methods described above. Crystals of CDDO-methyl ester were grown and submitted for single crystal structure analysis. The crystal structure was determined by single crystal X-ray diffraction. The proposed structure of CDDO-methyl ester is shown in FIG. 1.

[0168] The tetragonal cell parameters and calculated volume are: $a=14.21620(10)$ Å, $b=14.21620(10)$ Å, $c=81.5875(12)$ Å, $\alpha=90.00^\circ$, $\beta=90.00^\circ$, $\gamma=90.00^\circ$, $V=16488.9(3)$ Å³. The molecular weight of CDDO methyl ester is 505.70 g/mol with $Z=24$ resulting in a calculated density of 1.222 g cm⁻³. The space group was determined to be $P4_32_12$ (no. 96). A summary of the crystal data and crystallographic data collection parameters is provided in Table 10.

[0169] The quality of the structure obtained is high, as indicated by the R-value of 0.051 (5.1%). Usually R-values in the range of 0.02 to 0.06 are quoted for the most reliably determined structures.

[0170] An ORTEP drawing of a single CDDO methyl ester molecule is shown in FIG. 12. The asymmetric unit shown in FIG. 13 contains three CDDO methyl ester molecules. The molecules are the same as the proposed structure from FIG. 1.

[0171] Packing diagrams viewed along the a, b, and c crystallographic axes are shown in FIGS. 14-16, respectively. With no hydrogen bonds, the crystal structure includes numerous van der Waals interactions. The view down the crystallographic b axis (FIG. 15) highlights the helical nature of the packing arrangement of the tetragonal screw axis and the predicted BFDH morphology. The predicted morphology is in good agreement with the observed habit of the single crystal used in the data collection.

[0172] The calculated XRPD pattern of CDDO methyl ester, generated from the single crystal data, is provided in FIG. 17. The experimental XRPD pattern of CDDO methyl ester is shown in FIG. 18. Characteristic peaks for the Form A XRPD pattern are provided in Table 17. A comparison of the calculated and experimental XRPD patterns (FIG. 19) reveals all peaks in the experimental patterns are represented in the calculated XRPD pattern, indicating the bulk material is likely a single phase. The slight consistent shifting observed in peak location is likely due to the fact that the experimental powder pattern was collected at room temperature, and the single crystal data were collected at 150° K. Low temperatures are used in single crystal analysis to improve the quality of the structure.

[0173] In summary, the single crystal structure of CDDO methyl ester Form A was determined to confirm to the proposed molecular structure. The space group was determined to be $P4_32_12$ (no. 96). The structure of CDDO methyl ester consists of three molecules packed in a helical nature down the crystallographic b axis. All peaks in the experimental patterns are represented in the calculated XRPD pattern, indicating the bulk material is likely a single phase.

[0174] The thermal data for Form A are shown in FIG. 3. The DSC curve shows a baseline shift at approximately 157° C. and an endotherm with an onset temperature of approximately 222° C. (signal maximum at 224° C.). The event at 224° C. was confirmed as the melt by hot stage microscopy (FIG. 4). The thermogravimetry (TG) curve exhibits a negligible weight loss of 0.34% up to 150° C., followed by a weight loss of 1.2% from 150 to 210° C. Karl Fischer data shows the material to contain approximately 0.38% residual water, consistent with the initial weight loss observed by TG.

[0175] The DVS data indicate that Form A is not hygroscopic (FIG. 5). The material shows a negligible weight change throughout the experiment. The resulting material was analyzed by XRPD and is Form A.

[0176] SEM images are shown in FIG. 6. Several habits are observed, including pyramidal, tablet, and plate-like.

[0177] The physical stability of Form A at various conditions was investigated (Table 7). Samples stressed at either 25° C./60% RH or 40° C./75% RH for 7 days both showed a negligible weight change (0.6% loss and 0.2% gain, respectively) suggesting that Form A is not hygroscopic. Two samples were ground on a ball mill for approximately 20 minutes, one dry and one with a small amount of water. All samples were reanalyzed by XRPD and remained Form A. A sample was stressed at 195° C. for 15 minutes and showed a 2% weight loss. The XRPD of the resulting material is similar to that of Form A; however, an increase in the baseline noise is evident (FIG. 7).

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[0178] The solution NMR spectrum is shown in FIG. 8. The spectrum is consistent with the structure of CDDO methyl ester. Peaks at approximately 1.6 and 7.3 ppm are assigned to water and chloroform (due to exchange), respectively.

[0179] Form A is unsolvated and not hygroscopic, therefore, and it melts at approximately 228° C., based on observations of analyst during hot stage microscopy.

Example 4

Characterization of CDDO-Methyl Ester—Form A (Micronized)

[0180] Micronized Form A CDDO methyl ester was determined to be Form A by XRPD (FIG. 2, Table 1). Micronized material can be produced by conventional methodology, well known to the field, such as air jet milling. These findings appear to indicate that micronization does not affect Form A in order to alter its XRPD pattern.

Example 5

Characterization of CDDO-Methyl Ester—Form B

[0181] Form B material can be prepared from lyophilization, melt/quench, and several other evaporation experiments, as provided in Table 3.

[0182] The modulated DSC (MDSC) data are shown in FIG. 9. The reversible curve shows a glass transition temperature (T_g) at approximately 125° C. The non-reversible curve shows an exotherm with signal maximum at 195° C. and an endotherm with signal maximum at 223° C. The non-reversible events are most likely due to crystallization of the Form B material (exotherm) followed by the melt of the crystallized material (endotherm).

[0183] The physical stability of Form B material at various conditions was investigated (Table 9). Samples stressed at 22° C./97% RH, 40° C./75% RH, 80° C./0% RH, and 195° C./ambient RH remained Form B. Stressing the material at 200° C./ambient RH for 60 minutes produced Form A plus minor Form B material (FIG. 10).

[0184] The solution NMR spectrum is shown in FIG. 11. The spectrum is consistent with the structure of CDDO methyl ester. Peaks at approximately 1.6, 5.3, and 7.3 ppm are assigned to water, dichloromethane, and chloroform, respectively.

[0185] Form B is not hygroscopic, crystallizes to Form A at approximately 200° C., and has a glass transition temperature (T_g) of approximately 125° C.-130° C.

[0186] In summary, Form B is not hygroscopic. The MDSC data indicates that the glass transition temperature (T_g) of Form B is approximately 125° C.-130° C. Form B material crystallizes to Form A when stressed at approximately 200° C.

Example 6

Stability Studies for Form B CDDO-Methyl Ester and CDDO-Methyl Ester Polymer Excipient Dispersions

[0187] (i) Purified Form B Studies

[0188] Form B CDDO-methyl ester was subjected to varying stress conditions. Table 15 provides some of the results from these studies. Form B CDDO-methyl ester produced by a useful but less preferred embodiment of this invention, involving the use of ethyl acetate as solvent, shows considerable stability. Nevertheless, testing of Form B samples produced in the presence of ethyl acetate revealed the formation of Form A after 28 days of storage at temperatures of 60° C.

and above. By contrast, all samples produced in accordance with a preferred embodiment of this invention, Example 11 below being illustrative thereof, retained amorphous characteristics after stress testing under particularly rigorous conditions (see Table 15). These studies show the surprising stability of the Form B material, especially when it is prepared in accordance with the aforementioned, preferred embodiment.

[0189] In addition, Form B samples prepared under varying conditions were analyzed to determine whether they have similar chemical properties. As described above, that is, Form B samples were prepared by cryogrinding, melt quench, and spray drying methods. In addition, unmiconized Form B was micronized to produce micronized Form B. PDF analysis was performed on the samples (FIG. 28), which were determined to be glassy in nature.

[0190] (ii) Studies with CDDO-Me Dispersion Preparations

[0191] Studies were performed to compare the performance of different Form B: polymer solid dispersions produced by spray drying. The product attributes studied included stability and drug dissolution profile.

[0192] Different polymers were used at three active pharmaceutical ingredient (API) to polymer ratios (20:80; 40:60 and 60:40% w/w).

[0193] The following three polymers were selected:

[0194] Methacrylic Acid—Ethyl Acrylate Copolymer (1:1)

[0195] Copovidone [1-vinyl-2-pyrrolidone—vinyl acetate copolymer (3:2)]

[0196] Hypromellose Phthalate.

[0197] Solutions of CDDO-Me, either Form A crystalline material or Form B material, and the polymer chosen for study were dissolved in a suitable solvent at the appropriate weight ratios, typically to afford from 10-20% by weight of solids in solution. The solvent typically employed was acetone. The resulting solutions were spray dried by means of a laboratory scale spray dryer (BUCHI, model B-290), equipped with a two-fluid nozzle, using nitrogen as a drying and carrier gas. Carrier gas inlet temperatures of 65-85° C. and outlet temperatures of 50-60° C. typically were employed. The solids were collected and the spray dried powders were post-dried under vacuum, further to reduce the levels of the organic solvents.

[0198] The spray dried powders were analyzed for the level of residual organic solvents, glass transition temperature (T_g) and bulk density. After post-drying the powders were also analyzed for purity, water content, average particle size, absence of crystalline material by X-ray powder diffraction (XRPD) and dissolution profile.

[0199] The physiochemical characteristics of the dispersions were evaluated after short term stressing (after 5 days, 40° C./75% RH) by XRPD and modulated differential scanning calorimetry (mDSC).

[0200] These studies found that the glass transition temperature, T_g , decreased during short term stressing, probably due to the uptake of moisture over the course of stressing. The decrease was more pronounced with the formulations having low CDDO methyl ester:polymer ratios. For samples before the short-term stressing, one or two endothermic transitions were observed at higher temperatures, although these temperatures are somewhat lower than the ones observed initially. The enthalpy associated with these transitions decreased as polymer content decreased. This is an indication that this transition is most probably related with the polymer and not with the melting of a crystalline form. In fact, for dispersions produced with PVP/VA, the temperature of this endothermic transition was similar to the one observed for pure excipient, as received from the manufacturer. In each

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case, the XRPD profile after stressing continued to be the characteristic halo pattern centered around $13.5^\circ 2\theta$, and no peaks associated with the crystalline form were detected.

[0201] Further spray drying studies on two formulation were conducted, using larger scale spray drying equipment. In these cases, a Niro pilot scale dryer model PSD-1 (mobile minor 2000) was employed. Also employed were equivalent nozzle and spray drying conditions to those described above. Tables 20 and 21 summarize the solutions prepared for spray drying and their characteristics following spray drying. The formulations showed a lower T_g , relative to pure Form B, due to the formulation including polymers with a lower T_g than Form B.

Example 7

Administration of CDDO Methyl Ester: Form B Versus Form A in Cynomolgus Monkeys

[0202] In phase 1 of this study, a quantity of hard gelatin capsules, containing pure micronized Form A CDDO-Me or pure micronized Form B CDDO-Me, were prepared by (i) adding an appropriately weighed quantity of the pure form of the drug substance to a size 1 hard gelatin capsule and then (ii) closing the capsule. No additional excipients were employed. Either CDDO methyl ester Form B or Form A was administered orally in a gelatin capsule to Cynomolgus monkeys (dose=4.1 mg/kg in all cases).

[0203] Administration of the CDDO methyl ester Form B provided a median exposure which was approximately 520% greater than the equivalent dose of CDDO methyl ester Form A in monkeys. Table 11 compares the individual animal drug exposures. A crossover design was implemented in this study to increase the 'n' value and to enhance data reliability. A wash-out period of 1 week was implemented during the crossover. FIG. 20 demonstrates the achieved plasma concentrations of both forms of CDDO methyl ester over time in the sampled population. FIG. 21 represents comparative CDDO methyl ester plasma concentrations of animals #505M and #507F. FIG. 22 represents comparative CDDO methyl ester plasma concentrations of animals #508F and #502M.

[0204] To assess further the comparative bioavailability of the oral dosage forms of CDDO-methyl ester, including those containing CDDO-methyl ester excipient dispersions, two additional study phases 2 and 3 were conducted, as detailed below. These studies included some of the CDDO-Me Form B—polymer dispersions described in Example 6. All formulations included commonly used formulation additives.

Phase 2.

[0205] An aqueous suspension of nanocrystalline CDDO-Me was produced, starting from a sample of micronized Form A material. A Retsch® Planetary Ball Mill model PM 400, containing zirconia balls of 2 mm average size, was charged with 25 gm of micronized CDDO-Me (average particle size distribution of 6.1 μm), 5 gm of docusate sodium, 1 gm of Tween 80, and 68.3 gm of water. Grinding was initiated at approximately 400 RPM and was continued for 2 hours. A particle size distribution (PSD) determination using a laser light granulometer indicated an average PSD of 0.37 μm was obtained. To this thick suspension were added 1 gm of microcrystalline cellulose and 0.2 gm of xanthan gum, with brief mixing, and the suspension was stored refrigerated.

[0206] The ball milled nano suspension was spray coated onto a dry excipient blend in a laboratory scale Aeromatic Strea 1 fluid bed, with the top spray assembly having a spray nozzle size of 0.4 mm. The inlet temperature was set at 55°C . The exhaust temperature range during spraying was 32 to 35°

C. The resulting granulation was dried for approximately 5 minutes, until the exhaust temperature reached 38°C . (Attachment 3-5). The composition of the coated materials is given below.

Theoretical Composition of the nanocrystalline 30 mg CDDO-Me Formulation			
Item#	Ingredient	%	mg/unit
1	CDDO-Me	5.7	19
2	Tween 80	0.12	0.4
3	DOS	1.14	3.8
4	Microcrystalline Cellulose in suspension	0.21	0.7
5	Xanthan Gum	0.03	0.1
6	Microcrystalline Cellulose in granulation	24.4	81.55
7	Starch Pregel	58.5	195.3
8	Povidone K29/32	0.5	1.75
9	Crospovidone	8.4	28
10	Colloidal Silicon Dioxide	0.5	1.75
11	Magnesium Stearate	0.5	1.75
Total:		100.0	334.1

[0207] The resulting dry granulation was submitted for HPLC analysis to determine the assay of active ingredient. That assay was determined to be 14.4% (wt/wt), substantially higher than the theoretical value (5.7%). Based on the HPLC analysis, capsules were filled so as to afford a net CDDO-Me content of 30 mg.

[0208] The crystalline micronized Form A and amorphous, micronized Form B formulations were produced by a conventional dry powder blending process, using, as additives, microcrystalline cellulose, pregelatinized starch, crospovidone (functioning as a disintegrant), colloidal silicon dioxide, and vegetable grade magnesium stearate. Micronized CDDO-Me Form A of average PSD 6.1 μm was used for the Form A formulation, while micronized CDDO-Me Form B of average PSD 10.8 μm was employed for the corresponding CDDO-Me Form B formulation. The table below presents the quantitative composition of both formulations.

Form A and Form B 30 mg capsule Quantitative Composition		
Identity	% w/w	mg per capsule
CDDO-Me (Micronized)	18.18	30.0
Microcrystalline Cellulose	18.55	30.6
Pregelatinized Starch	53.45	88.2
Crospovidone	8.72	14.4
Colloidal Silicon Dioxide	0.55	0.9
Magnesium Stearate (Vegetable Grade)	0.55	0.9
Total Capsule Contents	100.00	165 mg

[0209] Each of 15 male Cynomolgus monkeys, *Macaca fascicularis*, received a single oral administration of CDDO-methyl ester (3 different formulations, 5 monkeys per formulation) at a target dose level of 10 mg/kg. The monkeys ranged from 1-3 years in age and 2.5-3.5 kg in size. Blood samples were collected up to 72 hours post-dose.

Phase 3:

[0210] The CDDO-Me excipient dispersions described in Example 6 were further formulated by a conventional dry powder blending process, using microcrystalline cellulose, lactose monohydrate, crospovidone (functioning as a disin-

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tegrant), and sodium lauryl sulfate as additives. The quantitative composition of each formulation appears below.

Composition of CDDO-Me excipient dispersions blended with additives in capsule formulation			
Component	40% CDDO-Me/ 60% Methacrylic acid copolymer Type C	60% CDDO-Me/ 40% PVP/VA	60% CDDO-Me/ 40% HPMC-P
CDDO-Me Dispersion	60.0%	50.0%	50.0%
Microcrystalline Cellulose	12.8%	16.0%	16.0%
Lactose monohydrate	20.0%	25.0%	25.0%
Croscopvidone	6.4%	8.0%	8.0%
Sodium lauryl sulfate	0.8%	1.0%	1.0%
Total Capsule Contents	100%	100%	100%
Fill mass	125 mg	100 mg	100 mg

[0211] After a suitable wash-out period (7 to 10 days), the same 15 male Cynomolgus monkeys used in Phase 2 each received a single oral administration of CDDO-methyl ester (3 different formulations, 5 monkeys per formulation) at a target dose level of 10 mg/kg. Blood samples were collected up to 72 hours post-dose.

[0212] The below chart summarizes each phase of the study.

Phase number	Route of administration	CDDO-Me formulation	Target dose level	Number of animals (males)
2	Oral (capsule)	Nanocrystalline Form A	30 mg/per animal (10 mg/kg)	5
		Amorphous micronized Form B		5
		Crystalline micronized Form A		5
3	Oral (capsule)	PVP/VA Form B	30 mg/per animal (10 mg/kg)	5
		HPMC-P Form B		5
		Methacrylic acid copolymer Type C-Form B		5

[0213] The mean intravenous and oral doses received by male Cynomolgus monkeys and mean concentrations of CDDO-methyl ester are summarized below:

Phase number	Formulation	Mean body weight (kg)	Mean dose (mg/kg)
2	PO (nanocrystalline Form A)	2.89 ± 0.267	10.5 ± 0.971
	PO (amorphous micronized Form B)	2.87 ± 0.177	10.5 ± 0.635
	PO (crystalline micronized Form A)	2.91 ± 0.202	10.3 ± 0.702
3	PO Form B (PVP/VA)	2.98 ± 0.311	10.2 ± 1.06
	PO Form B (HPMC-P)	2.93 ± 0.183	10.3 ± 0.627
	PO Form B (Methacrylic acid copolymer Type C)	2.93 ± 0.142	10.2 ± 0.486

PO Oral administration by capsule.

[0214] Gelatin capsule size 2 was used to deliver the formulations in phase 2 and gelatin capsule size 1 was used for delivery in phase 3. The net drug content in each capsule was

30 mg, corresponding to a 10 mg/kg dosage of drug, based on an assumption that each monkey weighed 3 kg. The capsule was attached to a gavage, the animal was gavaged, and the capsule was released from the end of the gavage by air pressure from an empty syringe. A small amount of water (approximately 10 mL) was given orally after the administration of the last capsule.

[0215] Serial blood samples (approximately 1 mL) were removed from the femoral vein or artery of each animal and transferred into tubes containing K₂-EDTA at each of the following time points (actual times were recorded):

[0216] Phase 2 Pre-dose, 1, 2, 4, 8, 16, 24, 48 and 72 h post-dose

[0217] Phase 3 Pre-dose, 1, 2, 4, 8, 16, 24, 48 and 72 h post-dose

[0218] All samples were thoroughly mixed following collection and placed on wet ice, prior to being refrigerated at approximately 4° C. CDDO-methyl ester concentrations in blood were analysed by HPLC-MS/MS.

[0219] The results are provided in Table 22 and in FIG. 30. For phase 2, Form B showed significantly better bioavailability to the two Form A formulations tested. Phase 3 results show that each of the CDDO-Me:polymer dispersion-based formulations had much higher bioavailability than either the micronized Form A or nanocrystalline Form A formulations. The methacrylic acid copolymer Type C and HPMC-P formulations showed the greatest bioavailability in the subject monkeys.

Example 8

Characterization of Hemibenzenate Form of CDDO-Methyl Ester

[0220] Various experiments, replicating the last recovery step from the synthesis of CDDO methyl ester, were performed. Honda et al., 2000. The objective was the isolation of crystalline material from a solution mixture of (10:1) benzene/acetone.

[0221] Approximately 100 mg of CDDO-methyl ester was dissolved in 300 µL of benzene/acetone (10:1) and filtered through a 0.2-µm nylon filter. The solution was then sonicated using an ultrasonic processor for 10 minutes and allowed to evaporate at room temperature in an uncapped vial overnight. A clear gel formed and 100 µL of benzene/acetone (10:1) was added. The solution was submitted to sonication on an ultrasonic processor for approximately 30 minutes. A white precipitate formed. The solids were allowed to air dry.

[0222] In other experiments, approximately 200 mg of CDDO-methyl ester was dissolved in 0.8 mL of benzene/acetone (10:1) and filtered through a 0.2-µm nylon filter. The

solution was then evenly divided into two 1-dram vials. Samples A and B were then allowed to fast evaporate at room temperature for a few hours. Sample A was capped and placed in a freezer. After the sample froze, the sample was allowed to thaw at room temperature. A small scratch was introduced using a spatula and the sample was allowed to evaporate at room temperature. White solids formed and were allowed to air dry. Sample B was capped, left at room temperature and was a clear solution after sitting at room temperature overnight. A small scratch was introduced using a spatula and the sample was allowed to evaporate at room temperature. White solids formed and were allowed to air dry.

[0223] Crystalline material, determined to be a hemibenzenate, was obtained from several of these experiments. As described above, minor disturbances, such as sonication or merely introducing a small scratch within the recovery vessel, will facilitate the crystallization of the benzene solvate (Table 12).

[0224] Characterization data of the hemibenzenate are summarized in Table 13. Characteristic peaks for the hemibenzenate XRPD pattern are provided in Table 19. The DSC curve exhibits a broad endotherm near 133° C., associated with ~7.0% of weight loss in the TG thermograph FIG. 23. The weight loss is likely due to the volatilization of benzene (see NMR discussion below), and corresponds to 0.5 moles of benzene for each mole of CDDO-methyl ester. The DSC endotherm observed near 223° C. most likely results from the melt of desolvated material.

[0225] These data distinguish previously isolated forms of CDDO-methyl ester from the present invention.

Example 9

Characterization of Novel Dimethanolate Form of CDDO-Methyl Ester

[0226] A CDDO-methyl ester dimethanol solvate was prepared according to the below procedure. Approximately 500 mg of CDDO-methyl ester was dissolved in 20 mL of methanol at 60° C. The solution was then slowly added to 20 mL of cold methanol at -10° C. with agitation. White solids were collected by vacuum filtration and then stored in a freezer.

[0227] Characterization data are summarized in Table 14. Characteristic peaks for the dimethanolate XRPD pattern are provided in Table 18.

[0228] The DSC curve shows a broad endotherm near 102° C., associated with ~11% of weight loss in the TG thermograph (FIG. 24). The TGIR data confirms the weight loss is due to volatilization of ~2.0 moles of methanol (FIG. 25). The resulting material from the TGIR experiment was recovered and was amorphous by XRPD (FIG. 26). A baseline shift at approximately 130° C., a broad exotherm near 203° C. followed by a sharp endotherm (onset: 223° C.) are also observed in the DSC curve. These events are most likely indicative of the Tg of the amorphous material (Form B) obtained through the desolvation of the dimethanol solvate followed by crystallization of the amorphous material to Form A and melting of that crystalline material.

[0229] The solution proton NMR spectrum was obtained. The chemical assignments were not performed; however it appears consistent with the chemical structure of CDDO-methyl ester. The peaks at ~3.51 ppm are assigned to methanol and correspond to ~1.7 moles. This result is consistent with the thermal data above.

Example 10

Clinical Studies with CDDO-Methyl Ester

[0230] CDDO-Me, formulated using micronized Form A, was selected for clinical development and first tested in a

Phase I safety-oriented study in patients with advanced cancer who had failed to respond adequately to prior therapies. In this Phase I dose-escalation trial, CDDO-Me was administered to 21 adult patients with various forms of advanced (metastatic) cancer. Patients were administered daily doses of CDDO-Me capsules at doses ranging from 5 to 900 mg/day (specifically 5, 10, 20, 40, 80, 150, 300, 600, or 900 mg/day). CDDO-Me was administered in "cycles" which were repeated until the patient experienced unacceptable toxicity or showed evidence of disease progression. In this study, one cycle of CDDO-Me consisted of 21 consecutive days of dosing followed by a 7-day rest period after which the patient was eligible to start the next cycle.

[0231] Both the safety and anti-tumor activity of CDDO-Me were reviewed. In addition, the biological effects of CDDO-Me were characterized. CDDO-Me was very well tolerated in these patients, with no significant drug-related adverse events reported. Several patients (approximately 75% of evaluable patients) were considered to have stable disease (based on standard radiological and clinical criteria) at the first evaluation point following completion of the second treatment cycle. Patients who were found to have evidence of progressive disease before completing the second cycle were not formally evaluated, and were not included in the group of evaluable patients. Five patients, including patients with melanoma and renal cell cancer, continued to show stable disease, some with evidence of regression of individual tumor lesions, after four cycles of treatment. Four patients were considered to have stable disease after at least six cycles of treatment. No new metastases developed in any patient receiving a dose of at least 40 mg CDDO-Me per day according to the prescribed schedule.

[0232] Based on the known anti-inflammatory properties of CDDO-Me, circulating inflammatory cytokines were evaluated in patients in the Phase I trial. At doses as low as 5 mg/day, there was a reduction of several circulating pro-inflammatory cytokines and chemokines including MMP-9, TNF α , IL-8, and VEGF. In particular, TNF α , which is known to play a significant role in the inflammatory process of diseases such as rheumatoid arthritis, was reduced substantially or to below detectable limits in 3 patients with elevated baseline TNF levels (one patient each at treatment doses of 10, 20, and 40 mg per day). Unlike anti-TNF monoclonal antibodies, which bind to and render targets inactive, CDDO-Me reduces production of TNF α and resultant circulating levels of TNF α .

[0233] Additionally, phase 2 gene products, which include antioxidant and detoxification enzymes, have been monitored in peripheral blood mononuclear cells of patients in the Phase I study. Significant induction of NQO1 (NAD(P)H:quinone oxidoreductase), a marker of phase 2 transcriptional activity, has been seen at doses of 10 mg/day and above.

[0234] Tumor biopsy data from several patients, taken after two cycles of treatment with CDDO-Me, indicated pronounced reductions in tumor tissue levels of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and phosphorylated STAT3 (pSTAT3). High levels of expression of each of these proteins are known to be correlated with tumor progression and poor clinical outcomes. Tumor biopsy data in several patients also indicated a pronounced degree of tumor cell death after two cycles of treatment with CDDO-Me. Levels of serum creatinine were significantly lower on day 21, compared to the pre-treatment baseline level, in more than 80% of the patients in this study. A number of patients who continued on treatment for multiple cycles showed continuing reductions in serum creatinine. Since serum creati-

nine is a widely used indicator of renal function, these observations indicate that treatment with CDDO-Me improves kidney function.

[0235] These studies provide data in human cancer patients showing the beneficial effect of CDDO-methyl ester on patients suffering from cancer. The data further indicate that CDDO-Me is likely to have clinically useful effects in patients suffering from other inflammation-related diseases, including renal dysfunction.

Example 11

Large Scale Production of Form B Using a Dimethanol Solvate Intermediate

[0236] One kilogram of Form A CDDO-Me was dissolved in 60±5° C. methanol to afford a complete solution. The resulting hot solution of CDDO-Me was added to a vessel containing cold -5° C. to -15° C. methanol, while maintaining agitation and a temperature of -5° C. to -15° C. throughout the addition. The resulting suspension of crystalline dimethanol solvate of CDDO-Me was filtered. The resulting solids, which displayed an XRPD pattern consistent with that presented in FIG. 26 (prior to TGIR analysis), were dried in an oven at 70±5° C. Drying was continued until the XRPD profile displayed no reflections characteristic of crystalline substance. The resulting XRPD amorphous CDDO-Me solids were passed through a sieve and packaged. Product recovery ranges from 65-95%.

Example 12

Cryoground Form A and Form B

[0237] Form A was cryoground and analyzed. The measured x-ray data of the sample obtained through cryogrounding (2 hours) showed some broadening in the peak at approximately 13.5° 2θ. PDF analysis of cryoground Form A produced results similar to the Form B analysis. These results suggest that the cryoground Form A is a glassy material and that cryogrounding can provide an alternative method for producing Form B.

[0238] Form B was cryoground and analyzed. The measured x-ray data of the sample obtained through cryogrounding (1 hour) was similar to the starting Form B material. These results indicate that Form B is stable and does not change Form due to cryogrounding.

TABLE 1

Sample Information		
SAMPLE INFORMATION	DESCRIPTION	XRPD RESULT
Unmicronized	White powder	A
micronized	—	A

TABLE 2

Approximate Solubilities of CDDO methyl ester	
SOLVENT	SOLUBILITY (mg/mL) ^a
Acetone	70
ACN (acetonitrile)	66
DCM (dichloromethane)	>194
1,4-Dioxane	21
EtOH (ethanol)	4

TABLE 2-continued

Approximate Solubilities of CDDO methyl ester	
SOLVENT	SOLUBILITY (mg/mL) ^a
EtOAc (ethyl acetate)	36
Hexanes	<1
(10:1) Isopropanol/water	4
MeOH (methanol)	8
MTBE (tert-butyl methyl ether)	4
THF (tetrahydrofuran)	97
Toluene	38
Water	<0.1

^aSolubilities are calculated based on the total solvent used to give a solution; actual solubilities may be greater because of the volume of the solvent portions utilized or a slow rate of dissolution. Solubilities are rounded to the nearest mg/mL.

TABLE 3

Crystallization Experiments on CDDO methyl ester			
SOLVENT	CONDITIONS ^a	HABIT/ DESCRIPTION	XRPD RESULT
Acetone	FE	White chunks	A
	SE	White broken glass	Form B
	SC	No solid	—
(3:10) Acetone/hexanes	FE	White broken glass	A + Form B
	SE	White chunks	A
	SC	Small white blades	A
(7:5) ACN/water	Rotovap	White broken glass	Form B
	FE	Broken glass	Form B
	SE	White broken glass	Form B
(1:5) DCM	SC	No solid	—
	FE	White broken glass	Form B
	SE	White broken glass	Form B
(1:5) DCM/hexanes	FD	Fluffy white solid	Form B
	FE	White chunks	A
	SE	White irregulars	A
EtOH	SC	Small white tablets	A
	FE	White broken glass	Form B
	SE	White broken glass	Form B
EtOAc	SC	No solid	—
	FE	White broken glass	Form B
	SE	White broken glass	Form B
(10:1) IPA/water	SC	Small white tablets	A
	FE	White chunks	A
	SE	White tablets, irregulars	A
MeOH	SC	Pyramidal tablets	A ^b
	FE	White broken glass	Form B
	SE	White broken glass	Form B
MTBE	SC	No solid	—
	FE	White broken glass	Form B
	SE	White broken glass	Form B
THF	SC	No solid	—
	FE	White broken glass	Form B
	SE	White broken glass	Form B
(1:2) THF/hexanes	SC	No solid	—
	FE	White chunks	Form B
	SE	White broken glass	Form B
Toluene	SC	No solid	—
	FE	White chunks	Form B
	SE	White broken glass	Form B
—	SC	No solid	—
	Melt/quench	Clear broken glass	Form B

^aFE = fast evaporation, SE = slow evaporation, SC = slow cool, temperatures are approximate, FD = freeze dry, rotovap = rotary evaporation

^bsingle crystal structure determination

TABLE 4

Slurry Experiments of CDDO methyl ester			
SOLVENT	TIME (DAYS)	HABIT/DESCRIPTION	XRPD RESULT
(10:1) IPA/water	7	White bits	A
MTBE	10	White chunks	A
MeOH	10	White chunks	A

TABLE 5

Cold Precipitation Experiments of CDDO methyl ester			
SOLVENT	ANTISOLVENT	HABIT/DESCRIPTION	XRPD RESULT
Acetone	Hexanes	No solid	—
	Water	White solid	A
DCM	Hexanes	No solid	—
THF	Water	White solid	A

TABLE 6

Characterization Data of Form A	
ANALYTICAL TECHNIQUE	RESULTS ^a
XRPD	A
DSC	Baseline shift 157° C., endotherm 224° C.
TGA	0.3% weight loss up to 150° C. 1.2% weight loss from 150 to 210° C.
KF	0.38%
Hot Stage	28.9: begin of heating 150.1: — 176.4: — 200.2: no changes 225.9: melting 227.9: melt point
MB	Negligible weight change throughout experiment
Post MB XRPD	A
SEM ^b	Pyramidal, tablets, and plates
NMR (CDCl ₃)	Consistent with structure

^aall temperatures are in° C., endo = endotherm
^bresults based on images taken

TABLE 7

Stress Studies of Form A		
CONDITIONS ^a	HABIT/ DESCRIPTION	XRPD RESULT
25° C./60% RH 3 days: 1.5% loss 7 days: 0.6% loss	White powder	A
40° C./75% RH 3 days: 1.4% loss 7 days: 0.2% gain	White powder	A
195° C. ^b 15 minutes: 2% loss	White powder	A
20 min grind, dry	White powder	A
20 min wet grind, water	White powder	A

TABLE 8

Characterization Data of Form B CDDO methyl ester	
ANALYTICAL TECHNIQUE	RESULTS ^a
XRPD	Form B
MDSC	Tg ~125° C.
NMR (CDCl ₃)	Consistent with structure

TABLE 9

Stress Studies of Form B CDDO methyl ester		
CONDITIONS ^a	HABIT/DESCRIPTION	XRPD RESULT ^c
22° C./97% RH 7 days	Broken glass	Form B
40° C./75% RH 8 days	Broken glass	Form B
80° C./0% RH ~24 hours	White broken glass, clear rounded chunks	Form B
195° C. ^b 5 minutes	White fines	Form B
200° C. ^b 60 minutes	White birefringent bits	A + Form B (min)

^aRH = relative humidity, Tg = glass transition temperature

^bAmbient lab humidity was measured as 74% RH

^cmin = minor

TABLE 10

Crystal Data and Data Collection Parameters for Form A	
formula	C ₃₂ H ₄₃ NO ₄
formula weight	505.70
space group	P 43 21 2 (No. 96)
a, Å	14.21620 (10)
c, Å	81.5875 (12)
V, Å ³	16488.9 (3)
Z	24
d _{calc} , g cm ⁻³	1.222
crystal dimensions, mm	0.01 × 0.01 × 0.00 ^b
temperature, K	150.
radiation (wavelength, Å)	Mo K _α (0.71073)
monochromator	graphite
linear abs coef, mm ⁻¹	0.074
absorption correction applied	empirical ^a
transmission factors: min, max	0.9995, 0.9999
diffractometer	Nonius KappaCCD
h, k, l range	-15 to 15 -10 to 10 -86 to 72
2θ range, deg	4.08-44.43
mosaicity, deg	0.32
programs used	SHELXTL
F ₀₀₀	6576.0
weighting	1/[σ ² (F _o ²) + (0.0176P) ² + 0.0000P]
where P = (F _o ² + 2F _c ²)/3	
data collected	46742
unique data	9168
R _{int}	0.093
data used in refinement	9168
cutoff used in R-factor calculations	F _o ² > 2.0σ(F _o ²)
data with I > 2.0σ(I)	5421
number of variables	1024
largest shift/esd in final cycle	0.00
R(F _o)	0.051
R _w (F _o ²)	0.085
goodness of fit	1.045

^aOtwinowski Z. & Minor, W. Methods Enzymol., 1997, 276, 307.

^bThe crystal dimensions are approximate

TABLE 11

Summary of the area under the plasma concentration (AUC) versus time in all animals studied (n = 8) CDDO METHYL ESTER FORMULATION STUDY - PARENT COMPOUND			
Monkey subject # *	Form A AUC (ng/ml * hr)	Form B AUC (ng/ml * hr)	% Form B over Form A
501M	37.5	203.4	542
502M	28.3	204.7	723
503F	21.3	107.3	504
504F	N/A	175	N/A
505M	60.4	158.3	262
506M	N/A	N/A	N/A
507F	57.3	164	286
508F	37.3	228	611
Mean	40.4	177.2	439
Median	37.4	175.0	523
Range (min-max)	21-60.4	158-228	262-723%

N/A = not determined.

TABLE 14

Characterization of CDDO-methyl ester Dimethanol Solvate	
ANALYTICAL TECHNIQUE	RESULTS
XRPD	Dimethanol solvate
DSC, 10° C./min	Endotherm 102° C. Baseline shift at 130° C. Broad exotherm 203° C. Endotherm 225° C. (onset: 223° C.)
TG, 10° C./min	11% weight loss up to 110° C. Corresponds to ~2.0 moles of methanol
TGIR, 20° C./min	9.8% weight loss up to 110° C. Corresponds to ~1.7 moles of methanol Volatile component identified as methanol
Post TGIR	X-ray amorphous
XRPD	
IR	Consistent with structure
¹ H NMR (CDCl ₃)	Consistent with structure ~1.7 moles of methanol evident

TABLE 12

Crystallization Experiments of CDDO-methyl ester.			
SOLVENT	CONDITIONS ^a	DESCRIPTION	XRPD RESULT
10:1 Benzene/acetone	RE, room temperature	White solids	X-ray amorphous
10:1 Benzene/acetone	FE followed by drying at 60° C.	Clear gel	—
10:1 Benzene/acetone	FE w/capillaries	Clear film	—
10:1 Benzene/acetone	FE, capped and put in a freezer over night, scratched the vial using a metal spatula and left to evaporate at room temperature	White solid	Benzene solvate
10:1 Benzene/acetone	FE, capped and sitting at room temperature over night, scratched the vial using a metal spatula and left to evaporate at room temperature	White solid	Benzene solvate
10:1 Benzene/acetone	FE w/ sonication	White solids	Benzene solvate

^aRE = rotary evaporation, FE = fast evaporation

TABLE 13

Characterization of CDDO-methyl ester Hemibenzene Solvate	
ANALYTICAL TECHNIQUE	RESULTS
XRPD	Hemibenzene solvate
DSC, 10° C./min	Endotherm 133° C. Endotherm 223° C. (onset 221° C.)
TG, 10° C./min	7.0% weight loss up to 160° C. Corresponds to 0.5 moles of benzene
¹ H NMR (CDCl ₃)	Consistent with structure ~0.5 moles of benzene evident

TABLE 15

Stress Studies of Micronized Form B CDDO-methyl ester				
Stress Conditions ^a	FORM B PRODUCED VIA EXAMPLE 11 PROCEDURE		FORM B PRODUCED BY ISOLATION FROM ETHYL ACETATE AND ETHANOL MIXTURES	
	XRPD Result	KF Result	XRPD Result	KF Result
80° C./P ₂ O ₅ , 14 d	Form B	NP	NP	NP
80° C./P ₂ O ₅ , 28 d	Form B	1.10	100% Form A	0.37
60° C./23% RH, 28 d	Form B	0.04	~50% Form A + Form B	0.02
40° C./48% RH, 28 d	Form B	0.00 ^b	Form B	0.39
25° C./75% RH, 14 d	Form B	NP	NP	NP
25° C./75% RH, 28 d	Form B	0.77	Form B	0.58

^aRH = relative humidity^bActual value was negative

TABLE 16

CDDO-Me Single Agent Anticancer Activity			
MODEL	AGENT/ROUTE	DRUG ACTIVITY	COMMENTS
NCI-H460 Lung Cancer Xenograft (nude rat)	CDDO-Me, p.o.	78% TGI ¹	Dose-dependent effects. CDDO-Me as effective as radiation
MCF-7/Her-2 Breast Cancer Xenograft (nude mouse)	CDDO-Me, i.v.	15% regression	Superior results achieved in tumors that over-express Her-2 ²
4T1 Syngeneic Breast Cancer Metastasis Model (mouse) - Early Treatment	CDDO-Me, i.v.	100% suppression	All animals remained tumor-free at sacrifice on Day 85 ³
4T1 Syngeneic Breast Cancer Metastasis Model (mouse) - Delayed Treatment	CDDO-Me, i.v.	67% suppression	33% of animals tumor-free at sacrifice on Day 95 ³
L3.6Pl. Pancreatic Cancer Xenograft (nude mouse)	CDDO-Me, i.v.	51% TGI	CDDO-Me outperformed Gemcitabine
DU-145 Prostate Cancer Xenograft (nude rat)	CDDO-Me, p.o.	77% TGI	Multiple schedules tested - TGI 60-77%

¹TGI = Tumor Growth Inhibition
Data presented is from doses at or below the MTD (defined as $\leq 10\%$ mortality and $\leq 20\%$ weight loss).
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TABLE 20

Solutions prepared for spray drying		
SOLID RATIO	40% Form B, 60% Polymer	60% Form B, 40% Polymer
POLYMER	Methacrylic acid copolymer Type C	HPMCP HP-55
SOLVENT	Acetone	Acetone
CONC. % W/W	10	10
TEMPERATURE° C.	-20	-20
OBSERVATIONS	Solution showed some haziness after the charge of the polymer. A sample of the solution was heated up to 35/40° C., but the haziness didn't disappear and some flocculation was observed..	The solution was completely clear after the charge of the polymer. The two runs done used the solution in the same day that it was prepared.

TABLE 21

Characteristics of tested formulations		
Polymer	Methacrylic acid copolymer, Type C	HPMCP HP-55
Tests		
Description	White solid	White solid
X-Ray Powder Diffraction	No crystalline peak detected	No crystalline peak detected
Water	1.50% w/w	—
DSC - Tg (inflection point)	106° C.	118° C.
Assay (by HPLC)	409% w/w (calculated on the anhydrous and solvent free basis)	59.4% w/w (calculated on the anhydrous and solvent free basis)
Total of impurities (HPLC)ti	1.14% area	0.42% area
Particle size (D50)	21 μ m	
Bulk density	0.18 g/cm ³ (calculated with 4 g of product)	0.19 g/cm ³ (calculated with 4 g of product)

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TABLE 22

Mean Concentrations of CDDO-Me in Plasma of Cynomolgus Monkeys in Phase 2 and 3 Samples						
Mean concentrations of CDDO-methyl ester (ng/mL)						
Time	PO Form A (nanocrystalline)	PO Form B (micronized)	PO Form A (crystalline micronized)	PO Form B (PVP-VA)	PO Form B (HPMCP)	PO Form B (Eudragit L100-55)
Pre-dose	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
5 min	NST	NST	NST	NST	NST	NST
10 min	NST	NST	NST	NST	NST	NST
15 min	NST	NST	NST	NST	NST	NST
30 min	NST	NST	NST	NST	NST	NST
1 h	1.53 ± 1.49	6.01 ± 5.67	0.619 ± 0.207	9.45 ± 3.64	9.94 ± 4.83	9.06 ± 4.38
2 h	3.00 ± 2.02	14.5 ± 9.63	0.505 ± 0.260	14.8 ± 3.04	24.1 ± 13.2	22.2 ± 11.7
4 h	3.52 ± 1.30	32.0 ± 11.6	1.15 ± 0.73	27.3 ± 3.79	50.7 ± 10.0	50.6 ± 19.3
8 h	8.56 ± 2.32	33.3 ± 8.44	4.31 ± 1.35	30.4 ± 10.8	40.0 ± 15.6	51.1 ± 8.72
16 h	7.90 ± 2.26	14.2 ± 5.68	5.39 ± 1.12	13.3 ± 3.92	15.4 ± 7.41	25.6 ± 13.9
24 h	4.40 ± 2.33	5.23 ± 1.86	3.05 ± 1.42	6.39 ± 2.02	5.79 ± 2.95	7.47 ± 4.10
36 h	NST	NST	NST	NST	NST	NST
48 h	0.649 ± 0.342	0.576 ± 0.175	0.419 ± 0.291	1.78 ± 1.61	0.983 ± 0.343	1.37 ± 0.626
72 h	0.312 ± 0.390	0.408 ± 0.368	0.0169 ± 0.0252	0.395 ± 0.319	0.448 ± 0.283	0.344 ± 0.461

NST No sample taken.

BLQ Below limit of quantification (1.00 ng/mL).

IV Intravenous administration.

PO Oral administration by capsule.

Values in bold italics calculated using all values presented in Appendix 3, consequently the mean may differ from means calculated where values that are BLQ are assumed to be zero.

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What is claimed is:

1. A glassy solid form of CDDO-methyl ester, having an x-ray powder diffraction pattern with a halo peak at approximately 13.5° 2θ, as shown in FIG. 2C, and a T_g.
2. A dimethanol solvate form of CDDO-methyl ester, having an x-ray powder diffraction pattern with characteristic peaks as shown in Table 18 and a DSC pattern as shown in FIG. 24.
3. A pharmaceutical composition in solid dosage form, comprising (i) a therapeutically effective amount of the glassy solid form of CDDO-methyl ester of claim 1 with (ii) an edible carrier.
4. A method for the production of the glassy solid form of CDDO-methyl ester of claim 1, comprising (a) preparing a dimethanol solvate form of CDDO-methyl ester, having an x-ray powder diffraction pattern with characteristic peaks as shown in Table 18 and a DSC pattern as shown in FIG. 24, and (b) drying the dimethanol solvate form to obtain said glassy solid form.
5. The glassy solid form of CDDO-methyl ester of claim 1, having a PDF spectrum with the characteristic peaks of FIG. 28 from about 5 Å to about 20 Å.
6. A therapeutic method that comprises administering an effective amount of the pharmaceutical composition of claim 3 to a subject suffering from a cancerous condition.
7. The method of claim 6, further comprising administering another anti-cancer drug to said subject.
8. The method of claim 7, wherein the other anti-cancer drug is gemcitabine and the cancerous condition is pancreatic cancer.
9. The glassy solid form of CDDO methyl ester of claim 1, having a T_g value in the range of about 120° C. to about 135° C.
10. The glassy solid form of CDDO methyl ester of claim 1, having a T_g value in the range of about 125° C. to about 130° C.

11. A pharmaceutical composition comprising (i) a therapeutically effective amount of CDDO-methyl ester and (ii) an excipient that is a glass former, such that said composition has a T_g.

12. A pharmaceutical composition of claim 11, wherein the excipient is selected from the group consisting of (A) a carbohydrate, carbohydrate derivative, or carbohydrate polymer, (B) a synthetic organic polymer, (C) an organic acid salt, (D) a protein, polypeptide, or peptide, and (E) a high molecular weight polysaccharide.

13. A pharmaceutical composition of claim 12, wherein the excipient is a synthetic organic polymer.

14. A pharmaceutical composition of claim 13, wherein the excipient is selected from the group consisting of a hydrox-

propyl methyl cellulose, a poly[1-(2-oxo-1-pyrrolidinyl)ethylene or copolymer thereof, and a methacrylic acid copolymer.

15. A pharmaceutical composition of claim 14, wherein the excipient is hydroxypropyl methyl cellulose phthalate ester.

16. A pharmaceutical composition of claim 14, wherein the excipient is PVP/VA.

17. A pharmaceutical composition of claim 14, wherein the excipient is methacrylic acid-ethyl acrylate copolymer (1:1).

18. A pharmaceutical composition of claim 13, wherein the excipient is copovidone.

* * * * *

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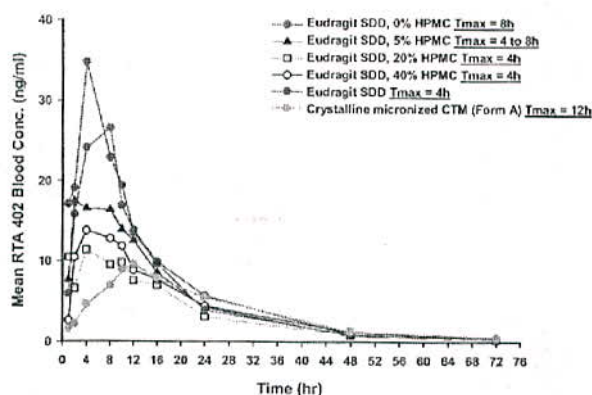
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(54) Title: DELAYED RELEASE, ORAL DOSAGE COMPOSITIONS THAT CONTAIN AMORPHOUS CDDO-ME

Figure 1

RTA 402 Eudragit SDD Formulations (5) 10 mg/kg vs RTA 402 Crystalline (Form A) 33 mg/kg
Blood Exposure in Cynomolgus Monkeys Following Single Oral,
Capsule Administrations (n=5)



(57) Abstract: Pharmaceutical formulations exhibit a desirably low C_{max} , among other properties, that contain particles of amorphous bardozone methyl, either in pure form or in the form of a solid dispersion, admixed with particles of a hydrophilic binding agent. Such formulations possess the advantage of higher oral bioavailability, relative to formulations based on the crystalline form of bardozone methyl.

**DELAYED RELEASE, ORAL DOSAGE COMPOSITIONS
THAT CONTAIN AMORPHOUS CDDO-ME**

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional
5 Application Serial No. 61/152,608, filed February 13, 2009, the content of which is
incorporated by reference into the present disclosure in its entirety.

BACKGROUND OF THE INVENTION

[0002] The synthetic triterpenoid bardoxolone methyl, also known as CDDO-Me and as
"RTA 402," has shown potent anti-inflammatory and anti-tumor properties in preclinical
10 studies and in human clinical trials. In particular, bardoxolone methyl has shown
significant anticancer activity in patients with advanced cancer, and has shown the ability to
improve measures of kidney function, insulin resistance, glycemic control, and systemic
cardiovascular disease in patients suffering from chronic kidney disease as a result of Type
2 diabetes.

15 [0003] In these studies bardoxolone methyl was administered orally in a crystalline form
("Form A"), once daily, at a variety of doses. In addition to the significant clinical efficacy
noted in these studies, Form A bardoxolone methyl showed an excellent tolerability profile
with very few drug-related side effects noted.

[0004] Pharmacokinetic data from these studies indicated, however, that Form A
20 bardoxolone methyl has relatively low oral bioavailability. Fortunately, a non-crystalline
form of bardoxolone methyl ("Form B") also has been identified, which shows markedly
superior oral bioavailability compared to Form A.

[0005] It is well understood that improved oral bioavailability is a desirable feature of a
drug formulation, since it reduces the per-dose cost of active material and is consistent with
25 the general medical principle of administering the lowest amount of a drug that is known to
produce the desired effect. Conversely, low aqueous solubility resulting in poor oral
bioavailability of potential drug candidates has been recognized as a significant challenge
facing the pharmaceutical industry.

[0006] In fact, an estimated 25-30% of compounds in early development have poor
30 bioavailability due to low solubility. The United States Food and Drug Administration has

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adopted a biopharmaceutics classification system (BCS) that classifies drugs intended for oral dosage according to solubility and membrane permeability. Drugs that are poorly soluble yet highly membrane permeable make up a substantial portion of drug candidates and are referred to as BCS class 2 drugs. For this class of drugs intended for oral dosage, improvements in effective bioavailability can occasionally be addressed by altering the solubility profile of the drug substance, either alone or via the use of functional excipients in an appropriate pharmaceutical composition.

[0007] Several techniques have evolved to improve the solubility of certain drug candidates that have the potential to be safe and effective. One such technique that has been explored is to formulate the drug using an amorphous form of the drug substance, either alone or in a polymer matrix. While improvements in aqueous solubility of amorphous forms over that of the corresponding crystalline forms of the drug substance have been documented, such systems are inherently unstable and may return to their thermodynamically more stable crystalline state. As a result, considerable research and experimentation often is conducted to define formulation systems that can yield formulations with acceptable shelf-life.

[0008] Because dissolution rates and solubility in physiological media are typically higher in the upper gastrointestinal system, formulations containing amorphous drug substances, if they can be developed, often behave differently *in vivo*, relative to those formulations containing the corresponding drug in a crystalline form. Formulations containing amorphous drug substances have been reported to produce bioavailability enhancements and have area-under-curve (AUC) values several-fold higher than formulations containing the corresponding crystalline form of the drug substance on an equivalent dose basis. While it is not unusual for amorphous forms of drugs, once absorbed into general circulation, to exhibit similar metabolism, distribution, and excretion profiles, the time to maximal plasma concentration (T_{max}) and the maximal concentration observed (C_{max}) often are altered markedly in formulations containing amorphous drugs compared to their crystalline counterparts.

[0009] If a drug exhibits toxicity or is associated with an increased frequency of adverse events above a certain limiting plasma concentration, then maintaining therapeutic plasma levels safely below such a limiting level may be of paramount importance. Thus, even if a drug has a broad therapeutic window and is otherwise safe and effective, the control of

C_{max} or T_{max} profiles may be important if the drug is to be administered chronically. More generally, if a particular plasma concentration profile is associated with a desirable profile of safety and efficacy, it is useful for alternative formulations containing the same active ingredient to produce a comparable plasma concentration profile.

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SUMMARY OF THE INVENTION

[0010] In view of the foregoing, the present invention provides, according to one of its aspects, a solid dosage form comprising (A) particles comprised of amorphous bardoxolone methyl admixed with (B) particles comprised of at least one hydrophilic binder, such as a cellulose-based excipient, where the particles (A) constitute a therapeutically effective amount of bardoxolone methyl. Illustrative of the class of suitable cellulose-based excipients are: C₃-C₁₀ alkyl hydroxymethyl cellulose, *e.g.*, methyl cellulose, ethyl cellulose, propyl cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, and cellulose acetate; aryl hydroxymethyl cellulose; and substituted aryl hydroxymethyl cellulose. Alternatively, the hydrophilic binder may be a naturally occurring carbohydrate polymer or an anionic polymer.

[0011] In one embodiment of the invention, the particles (A) consist essentially of amorphous bardoxolone methyl. In another embodiment, the particles (A) are comprised of a solid dispersion of amorphous bardoxolone methyl in a glassy matrix, which can be obtained, for example, as the product of a process that comprises spray drying a mixture of bardoxolone methyl and a methacrylic acid copolymer. Such spray drying can involve, for instance, using a 4:6 mixture of bardoxolone methyl and a methacrylic acid copolymer.

[0012] In accordance with this invention, the proportion of the hydrophilic binder in a solid dosage form as described here may be between about 1% and about 40% (w/w) of the total formulation, *e.g.*, between about 2% and about 20% (w/w), about 4% and about 10% (w/w), about 5% and about 7.5% (w/w), or about 7% and about 7.5% (w/w) or at about 7% (w/w) of the total formulation.

[0013] Formulations of the present invention exhibit modified C_{max} profiles relative to formulations that lack hydrophilic binding agents. More particularly, an inventive formulation produces a significantly lower C_{max} than that obtained with a comparable formulation containing the amorphous dispersion but lacking the hydrophilic binding agent.

The inventive formulation maintains the advantage of higher oral bioavailability compared to formulations based on the crystalline form of bardoxolone methyl.

BRIEF DESCRIPTION OF THE DRAWINGS

5 [0014] Figure 1 is a graphical representation of bioavailability data obtained via single-dose oral administration of different RTA-402 formulations to cynomolgus monkeys.

[0015] Figure 2 is a graphical representation of comparative pharmacokinetic data obtained, using cynomolgus monkeys, with different RTA 402 formulations containing 5.0% and 7.5% hydroxypropyl methylcellulose, respectively.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

10 [0016] Preclinical studies with various formulations containing an amorphous dispersion of bardoxolone methyl (Form B) indicate that its improved oral bioavailability was associated with significantly increased C_{max} relative to Form A material, as well as with an overall plasma concentration curve that differs markedly from that of Form A in equivalent doses. In view of the significant efficacy and excellent tolerability obtained with Form A
15 bardoxolone methyl in clinical studies, the present inventors sought to identify a formulation containing an amorphous dispersion of bardoxolone methyl that would maintain the advantage of improved oral bioavailability while producing a plasma concentration curve more closely resembling that associated with Form A. This would afford greater confidence that the efficacy and tolerability profile of such a formulation in
20 subsequent clinical studies would be consistent with that observed in studies using Form A material.

[0017] By virtue of this investigation, the present inventors discovered that a modified formulation of a solid dispersion of bardoxolone methyl, containing as an additive one or more hydrophilic binding agents, for example, cellulose-based binders, such as
25 hydroxypropyl methyl cellulose, showed the desired properties. These hydrophilic binding agents are believed to modulate dissolution rate, providing not only oral bioavailability that is several-fold greater than that of Form A material but also, following oral dosing, a lower C_{max} than previous Form B formulations. Consequently, a formulation of the invention yields an overall plasma concentration curve (PCC) that more closely resembles the PCC
30 associated with Form A material.

[0018] In animal studies, formulations based on a micronized solid dispersion of Form B admixed with hydrophilic binder showed significantly higher C_{max} values, relative to formulations containing equivalent doses of crystalline bardoxolone methyl. Thus, addition of the hydrophilic binder does not negate the superior bioavailability of such amorphous forms of bardoxolone methyl, in comparison with crystalline forms.

[0019] Qualitatively similar results should pertain as well for a formulation, according to the invention, of pure Form B particles admixed with particles of a hydrophilic binder. In this context, "pure" connotes the presence of amorphous bardoxolone methyl free of any material, including an excipient, that could affect the pharmaceutical properties of the drug.

This use of "pure" is intended not to denote absolute purity; rather, it comports with the normal standard of acceptable purity for a pharmaceutical agent. A synonymous phrasing in this regard qualifies particles of an inventive formulation as "consisting essentially of" Form B. In a solid dispersion that is comprised of Form B, a glass-forming excipient constitutes a significant percentage of the total material and is important in determining overall pharmacological properties.

[0020] Each non-crystalline form of bardoxolone methyl, whether pure Form B or a solid dispersion containing Form B combined with a glass-forming excipient, is characterized by a single glass transition temperature (T_g), which can be measured via differential scanning calorimetry. Each non-crystalline form of bardoxolone methyl also has a characteristic, broad halo peak, observed by X-ray powder diffraction (XRPD), which is indicative of the presence of an amorphous form.

[0021] A solid dispersion of bardoxolone methyl employed in accordance with one aspect of the present invention may be produced with any of various glass-forming materials, used as excipients. Thus, one embodiment of the invention is a formulation in which particles of such a solid dispersion of bardoxolone methyl are admixed with particles of a hydrophilic binder, optionally with particles of other excipients. The resulting admixture, when administered to a subject by oral dosing or other means, produces a modified plasma concentration curve compared to formulations containing the same amount of the solid dispersion of bardoxolone methyl but lacking the hydrophilic binder.

[0022] This modified plasma concentration curve is characterized by a lower C_{max} relative to the formulation that lacks the hydrophilic binder. By the same token, admixing particles of pure Form B bardoxolone methyl with particles of a hydrophilic binding agent,

pursuant to another aspect of the invention, yields similar effects on the plasma concentration curve. A lower C_{max} is manifested, that is, relative to an equivalent formulation lacking the hydrophilic binder.

5 [0023] A variety of preparative techniques can be used to produce solid dispersions of amorphous bardoxolone methyl, pursuant to this invention. Suitable in this regard, for example, is a variety of conventional thermal methods (e.g., hot melt extrusion), solvent methods, and thermal/solvent methods (e.g., spray drying or fluidized bed coating of granules).

10 [0024] Also suitable in accordance with the invention are ratios of bardoxolone methyl, the active ingredient, to the glass forming excipient that are other than the 4:6 ratio referenced below. As a function of the glass forming excipient and production methodology employed, suitable ratios can vary significantly, ranging, for example, between about 1:19 and about 2:1.

15 [0025] As noted above, any of a variety of glass forming excipients are suitable for use in the invention, so long as the given excipients can form a glassy solid matrix, having a glass transition temperature (T_g). Illustrative of such excipients are derivatives of cellulose (e.g., hydroxypropyl cellulose), acrylic acid derivatives and other synthetic polymers (e.g., polyvinyl pyrrolidone and copovidone), organic acid salts, and proteins and peptides (e.g., albumin and polyalanine).

20 [0026] A solid dosage form of the invention may be administered by other than oral dosing. These other, suitable administration routes include but are not limited to nasal, pulmonary, transmucosal, and transdermal delivery.

25 [0027] Solid dispersions of Form B (amorphous) bardoxolone methyl have exhibited superior oral bioavailability compared to formulations containing pure Form B bardoxolone methyl (data not shown). Still, both types of amorphous material have displayed dramatically improved oral bioavailability compared to crystalline forms of bardoxolone methyl. Accordingly, the present invention encompasses, in one of its aspects, a formulation that contains pure Form B bardoxolone methyl admixed with one or more hydrophilic binding agents, such that the admixture achieves an overall plasma
30 concentration profile similar to the formulations exemplified below. Such admixing does not yield a solid dispersion, regardless of whether the hydrophilic binder could serve as a glass-forming excipient in another context. This is so because the admixing process

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according to the invention does not involve steps, *e.g.*, dissolving both materials in a solvent and then spray-drying, that are required for the formation of a solid dispersion.

[0028] A formulation containing pure Form B bardoxolone methyl, pursuant to the invention, may require different proportions of hydrophilic binder to active, compared with
5 a formulation containing a solid dispersion of Form B, in order to achieve the desired plasma concentration profile. For instance, a lower amount of hydrophilic binder versus active ingredient might be required to compensate for the lower bioavailability of pure Form B material, relative to a solid dispersion containing a comparable amount of Form B material, as described above. More generally, either lower or higher proportions of
10 hydrophilic binder(s)-to-active might be required to achieve the desired results with pure Form B material, depending on the nature of the hydrophilic binding agent or agents employed and the effects of other excipients that may be present in the formulation.

[0029] A dosage form of the invention typically contains a therapeutically effective amount of amorphous bardoxolone methyl. In this regard, an amount that is
15 "therapeutically effective" is sufficient to activate the Nrf2 signaling pathway in circulating blood cells. See Ichikawa et al., (2009) PloS One, 4(12):e8391. More generally, a therapeutically effective amount can be determined empirically, by reference to a patient's clinical parameters.

[0030] To illustrate the invention, amorphous bardoxolone methyl-containing
20 compositions were prepared as a spray-dried dispersion (SDD). A solid dispersion of Form B, each of the SDD compositions was produced by spray-drying solutions that contained a 4:6 ratio of bardoxolone methyl (Form B) to a glass-forming excipient, methacrylic acid copolymer Type C, USP. A formulation was prepared by blending the resultant particles of a given SDD with a hydrophilic binding agent, such as hydroxypropyl
25 methyl cellulose, together with other excipients, as shown below in Table 1, followed by roller compaction of the blend, milling, and encapsulation of the granules thus obtained.

[0031] In general terms, the final product of this exemplary process was a mixture in granular form, each granule containing (i) particles of an amorphous dispersion containing Form B bardoxolone methyl, (ii) particles of the hydrophilic binder(s), and (iii) particles of
30 the other excipients. An analogous process could be used, varying in the nature of the starting material, *i.e.*, pure Form B rather than a solid dispersion containing Form B, to produce a mixture in granular form. In this instance, each granule would contain (1)

particles of pure Form B, (2) particles of the hydrophilic binder(s), and (3) particles of the any other excipient(s).

- [0032] As shown in Table 1, reference "formulation #1" (identified in Figure 1 as "Eudragit SDD" contained copovidone, a disintegration agent. Modifications to the reference formulation, each modification containing no copovidone, were produced and contained from 0% to 40% by weight of hydroxypropyl methylcellulose (HPMC), a representative of the cellulose-based hydrophilic binder subclass. Percentages of the excipients lactose and microcrystalline cellulose were adjusted downward accordingly (Table 1).

10 **Table 1. Composition of control formulation and formulation modifications containing hydroxypropylmethyl cellulose**

Components	#1	#2	#3	#4	#5
Bardoxolone methyl (SDD)	12.50%	12.50%	12.50%	12.50%	12.50%
Microcrystalline Cellulose	20.00%	29.00%	24.00%	29.20%	22.50%
Lactose Monohydrate	53.50%	53.50%	53.50%	33.30%	20.00%
Copovidone XL	9.00%	0.00%	0.00%	0.00%	0.00%
Sodium Lauryl Sulfate	3.00%	3.00%	3.00%	3.00%	3.00%
Colloidal Silicon Dioxide	1.00%	1.00%	1.00%	1.00%	1.00%
Magnesium Stearate	1.00%	1.00%	1.00%	1.00%	1.00%
HPMC	0.00%	0%	5.00%	20.00%	40.00%
Total	100.00%	100.00%	100.00%	100.00%	100.00%

[0033] Table 2 details the constituents of capsules that contain 50 mg of active ingredient, micronized crystalline (Form A) bardoxolone methyl. In Table 3 this formulation is designated "CTM."

15 **Table 2. Composition of bardoxolone methyl capsules containing micronized crystalline form of the drug substance**

Identity	% w/w	mg per capsule
Bardoxolone Methyl (Micronized)	18.18	50.0
Microcrystalline Cellulose	18.55	51.0
Pregelatinized Starch	53.45	147.0
Copovidone	8.72	24.0
Colloidal Silicon Dioxide	0.55	1.5
Magnesium Stearate	0.55	1.5
Total Capsule Contents	100.00	275 mg

[0034] Pharmacokinetic studies were carried out in fasted cynomolgus monkeys with an average body mass of 3 Kg. In animals treated with the formulations shown in Table 1, a

single capsule was administered by oral gavage. In animals treated with the micronized crystalline form as shown in Table 2, two capsules were administered by oral gavage. The dosage of the reference formulation and formulation modifications presented in Table 1 was approximately 10.0 mg / kg, while the dosage of the formulation composition containing the micronized crystalline form of the drug was 33.3 mg / kg.

[0035] Blood was withdrawn from each animal, at the time points indicated in Figure 1, and each sample was quantified for bardoxolone methyl content, using a validated LC/MS/MS bioanalytical test method. The pharmacokinetic data obtained are presented in Table 3.

10 Table 3. Pharmacokinetic parameter estimates from blood obtained following oral capsule administration of 10 mg/kg RTA 402 for each investigated Eudragit formulation and 33 mg/kg RTA 402 crystalline (Form A) CTM [mean (n=5); pharmacokinetic parameter estimates generated via non-compartmental analysis (WinNonlin™ software version 5.2)]

RTA 402 Oral Dose Formulation	ID in Table 1	C _{max} ng/ml	T _{max} hr	Cl/F L/hr/kg	Cl L/hr/kg	V _z /F L/kg	V _z L/kg	T _{1/2} hr	AUC _{0-72h}	%F
Eudragit SDD, 0% HPMC	#2	27.6	6.00	25.3	3.9	467	71	12.9	433	15.2
Eudragit SDD, 5% HPMC	#3	22.4	5.60	29.6	3.5	529	63	12.4	339	11.9
Eudragit SDD, 20% HPMC	#4	11.4	6.40	45.5	3.7	883	72	13.6	230	8.1
Eudragit SDD, 40% HPMC	#5	16.0	10.00	36.7	3.9	797	84	15.3	297	10.5
Eudragit SDD, control	#1	34.8	4.00	23.6	3.7	455	71	13.4	444	15.6
Crystalline micronized (Form A), CTM	NA	10.2	12.00	126.6	3.5	2706	76	14.8	258	2.8

15 CTM: clinical trial material, HPMC: hydroxypropyl methylcellulose, ND: not determined, SDD: spray dried-dispersion

Pharmacokinetic parameters defined:

20 C_{max}, maximum observed concentration; T_{max}, time of achieved maximum observed concentration; Cl/F, the apparent oral clearance assuming 100% bioavailability of drug; Cl, total body clearance of drug corrected for fraction of drug absorbed; V_z/F, the volume of distribution of drug assuming 100% bioavailability of drug, calculated from the terminal phase; V_z, volume of distribution of drug corrected for fraction of drug absorbed and calculated from the terminal phase; T_{1/2}, the estimated pharmacologic half-life of the drug, AUC_{0-72h}, is the estimated drug area under the curve from time zero through 72 hours of blood sampling; %F, percentage of drug absorbed relative to intravenous administration.

25 [0036] Figure 1 shows that the use of HPMC in a SDD containing Form B bardoxolone methyl (designated "RTA 402") alters the *in vivo* pharmacokinetic profile of the drug. For instance, increasing concentrations of HPMC lower the mean blood concentration of RTA

402 achieved from a given dose. Thus, a HPMC concentration of 20% w/w lowered the C_{max} by >50%, when compared to a control.

[0037] Based on the performance of the 5% HPMC formulation described above, in vitro dissolution studies were conducted with formulations of Form B with 2.5%, 5.0%, or 7.5% HPMC, as shown in the table below. The results of these studies suggested that higher percentages of HPMC were associated with slower dissolution rates.

Components	HPMC 2.5	HPMC 5.0	HPMC 7.5
Bardoxolone methyl SDD	12.50%	12.50%	12.50%
Microcrystalline Cellulose	30.00%	30.00%	30.00%
Lactose Monohydrate	50.00%	47.50%	45.00%
Copovidone XL	0.00%	0.00%	0.00%
Sodium Lauryl Sulfate	3.00%	3.00%	3.00%
Colloidal Silicon Dioxide	1.00%	1.00%	1.00%
Magnesium Stearate	1.00%	1.00%	1.00%
HPMC	2.5%	5.00%	7.50%
Total	100.00%	100.00%	100.00%

[0038] In light of these results, the 5.0% and 7.5% HPMC formulations were selected for comparative pharmacokinetic studies in cynomolgus monkeys, with a control in the form of the crystalline micronized Form A bardoxolone formulation described in Table 2. Each of the HPMC/Form B formulations was administered in capsules, via oral gavage, at doses of either 30 mg or 10 mg. The Form A control formulation was administered at a dose of 100 mg. Results (blood plasma concentration of RTA 402 vs. time) are shown in Figure 2.

[0039] An HPMC formulation containing Form B bardoxolone methyl also was prepared for human clinical studies. Bardoxolone methyl capsules were formulated at 15 mg strength. Table 4 depicts the components used on a per capsule basis. Table 5 presents the composition in a percentage basis.

[0040] As described above, the SDD contained 40% bardoxolone methyl active pharmaceutical ingredient (API). As a result, the use of 37.5 mg of SDD per capsule resulted in 15 mg of bardoxolone methyl per capsule.

[0041] In Tables 4 and 5, "SMCC" denotes silicified microcrystalline cellulose, a co-processed excipient comprised of compendial excipients. SMCC is listed in the FDA Inactive Ingredients Guide.

5 **Table 4 Batch formula for 15 mg strength bardoxolone methyl capsules on a per capsule basis**

Ingredients:	15 mg mg/Capsule
RTA-402 SDD (40% Dispersion of API)	37.5
SMCC (90LM)	120
Lactose Monohydrate	135
Hydroxypropyl methylcellulose	22.5
Silicon Dioxide Colloidal	3
Magnesium Stearate	3
Sodium Lauryl Sulphate	9
Total Capsule Fill Weight:	330 mg
Capsule Size	#1

Table 5 Batch formula 15 mg strength bardoxolone methyl capsules on percentage basis

Ingredients:	15 mg mg/Capsule
RTA-402 SDD (40% Dispersion of API)	11.36%
SMCC (90LM)	36.36%
Lactose Monohydrate	40.91%
Hydroxypropyl methylcellulose	6.82%
Silicon Dioxide Colloidal	0.91%
Magnesium Stearate	0.91%
Sodium Lauryl Sulphate	2.73%
Total	100.00%

- 10 [0042] To demonstrate that the Form B formulations of the present invention can maintain high bioavailability and achieve the desired PCC in humans, clinical pharmacokinetic studies were carried out in healthy volunteers. The volunteers received a single dose of either 150 mg crystalline bardoxolone methyl (Form A; 3 50 mg capsules) or 30 mg amorphous bardoxolone methyl (Form B; 2 15 mg capsules). Repeated blood samples were
- 15 subsequently taken and analyzed for plasma concentrations of the drug. After a 10-day washout period, each patient was given the form of the drug that was not administered the first time. A plasma concentration profile was measured again for each patient after the second treatment.

Table 6 Summary of pharmacokinetic parameters of the clinical testings

Patient entry no.	Treatment Period	Dose form	Dose (mg)	t _{max} (h)	C _{max} (ng/mL)	C _{48 h} (ng/mL)	AUC _{0-48h} (ng•h/mL)
101	1	Crystalline	150	30.0	1.89	1.31	61.0
102	2			24.0	1.89	1.57	51.6
103	2			24.0	1.10	0.59	31.9
104	1			30.0	2.19	0.87	51.7
105	1			24.0	0.73	0.30	22.5
106	2			48.0	0.79	0.79	22.7
	Mean			30.0	1.43	0.91	40.2
	SD			9.3	0.63	0.47	16.6
	CV(%)			31.0	44.28	51.50	41.3
101	2	Amorphous	30	4.0	1.79	0.24	29.1
102	1			4.0	5.03	0.25	37.9
103	1			2.0	3.83	0.27	49.1
104	2			8.0	3.43	0.47	55.4
105	2			2.0	1.98	0.15 ^a	19.2
106	1			2.0	4.13	0.25	30.9
	Mean			3.6	3.68	0.28	38.5
	SD			2.6	1.12	0.12	14.4
	CV(%)			72.4	30.38	42.13	37.4

^a Less than the limit of detection

5 [0043] As shown in Table 6, the Form B formulation showed higher bioavailability while achieving an overall exposure profile similar to the crystalline formulation, as measured by the 48 hr AUC values.

10 [0044] In above-described, exemplary compositions of the invention, HPMC illustrates the subclass of cellulose-based binders, including other C₃-C₁₀ alkyl and aryl substituted cellulose derivatives, that are suitable for use in this context. This subclass of the larger category of hydrophilic binding agents is illustrated as well by methyl cellulose, ethyl cellulose, propyl cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, and cellulose acetate.

15 [0045] For purposes of the present invention, preferred as binding agents are alkyl cellulose derivatives, such as hydroxypropyl methyl cellulose, which is commercially available in various molecular weight ranges. Other hydrophilic binding agents also may be

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used, such as: (a) naturally occurring carbohydrate polymers, e.g., starch and pregelatinized starch; (b) anionic polymers, e.g., acrylic acid homopolymers cross-linked with allyl sucrose or allyl pentaerythritol, (c) polymers of lactic acid or copolymers of lactic and glutamic acid, (d) gelatin or modified gelatins; and (e) amino-substituted carbohydrate polymers, e.g.,
5 chitosan.

[0046] Without being bound to any particular mechanism or theory, the present inventors believe that the various hydrophilic binding agents discussed above alter the pharmacokinetic profile of amorphous bardozone methyl in at least three ways, each contributing to a slower dissolution. First, the hydrophilic material(s), illustrated by HPMC,
10 serves as a binder during the roller compaction process, as described above, to hold the resultant particles together and to form a stronger binding among those primary particles. As a consequence, during dissolution the granules formed by the roller compaction disintegrate more slowly than would be the case otherwise. Second, during dissolution the binder agent or agents form a viscous gel that adheres the Form B-containing particles (and
15 the granules themselves) together, thus further slowing disintegration. Third, the aforementioned viscous gel increases local viscosity in the presence of the dissolution medium. The increase in local viscosity slows diffusion of drug and, hence, dissolution as well.

WHAT IS CLAIMED IS:

1. A solid dosage form comprising (A) particles comprised of amorphous bardoxolone methyl admixed with (B) particles comprised of at least one hydrophilic binder, wherein said particles (A) constitute a therapeutically effective amount of bardoxolone methyl.
5
2. The solid dosage form of claim 1, wherein the hydrophilic binder is a cellulose-based excipient.
3. The solid dosage form of claim 1, wherein said particles (A) are comprised of a solid dispersion of amorphous bardoxolone methyl in a glassy matrix.
- 10 4. The solid dosage form of claim 3, wherein said particles (A) are the product of a process that comprises spray drying a mixture of bardoxolone methyl and a methacrylic acid copolymer.
5. The solid dosage form of claim 4, wherein said process comprises spray drying a 4:6 mixture of bardoxolone methyl and a methacrylic acid copolymer.
- 15 6. The solid dosage form of claim 2, wherein the cellulose-based excipient is selected from the group consisting of C₃-C₁₀ alkyl hydroxymethyl cellulose, aryl hydroxymethyl cellulose, and substituted aryl hydroxymethyl cellulose.
7. The solid dosage form of claim 5, wherein the cellulose-based excipient is a C₃-C₁₀ alkyl hydroxymethyl cellulose selected from the group consisting of methyl cellulose, ethyl cellulose, propyl cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, and cellulose acetate.
20
8. A solid dosage form of claim 6, wherein the cellulose-based excipient is hydroxypropyl methyl cellulose.
9. The solid dosage form of claim 1, wherein the hydrophilic binder is a naturally
25 occurring carbohydrate polymer.

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10. The solid dosage form of claim 1, wherein the hydrophilic binder is an anionic polymer.
11. The solid dosage form of claim 1, wherein said particles (A) consist essentially of amorphous bardoxolone methyl.
- 5 12. The solid dosage form of claim 1, wherein the proportion of the hydrophilic binder is between about 1% and about 40% (w/w) of the total formulation.
13. The solid dosage form of claim 12, wherein the proportion of the hydrophilic binder is between about 2% and about 20% (w/w) of the total formulation.
- 10 14. The solid dosage form of claim 12, wherein the proportion of the hydrophilic binder is between about 4% and about 10% (w/w) of the total formulation.
- 15 15. The solid dosage form of claim 12, wherein the proportion of the hydrophilic binder is between about 5% and about 7.5% (w/w) of the total formulation.
16. The solid dosage form of claim 12, wherein the proportion of the hydrophilic binder is between about 7% and about 7.5% (w/w) of the total formulation.
- 15 17. The solid dosage form of claim 12, wherein the proportion of the hydrophilic binder is about 7% (w/w) of the total formulation.
18. The solid dosage form of any of claims 12-17, wherein the hydrophilic binding agent is hydroxypropyl methylcellulose.

Figure 1

RTA 402 Eudragit SDD Formulations (5) 10 mg/kg vs RTA 402 Crystalline (Form A) 33 mg/kg
Blood Exposure in Cynomolgus Monkeys Following Single Oral,
Capsule Administrations (n=5)

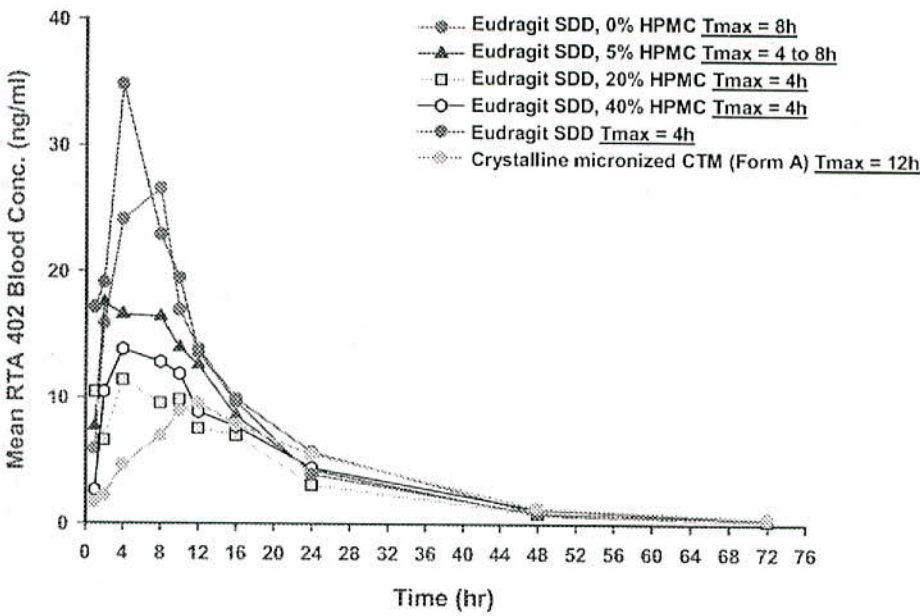
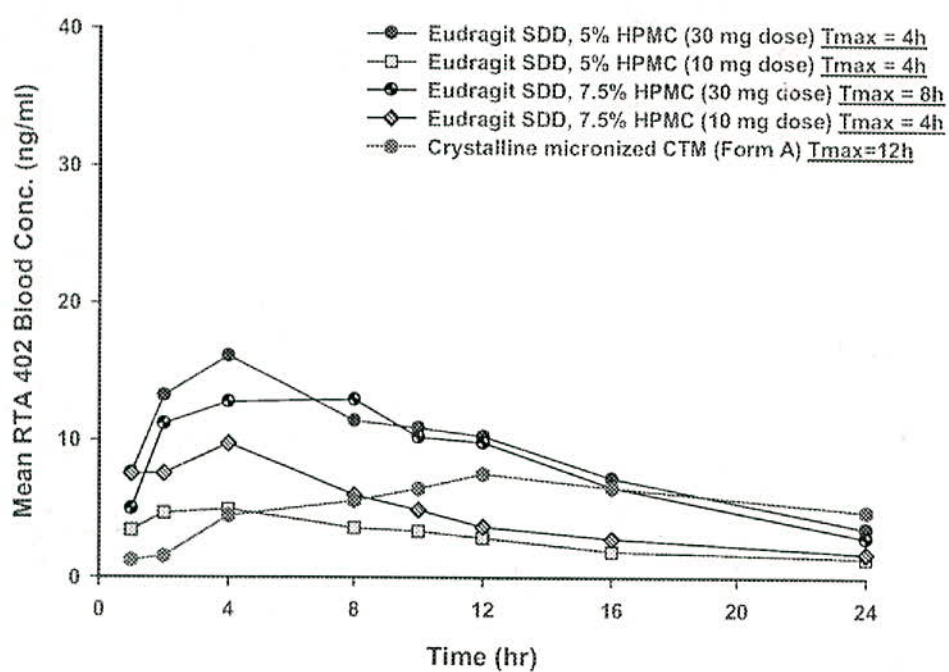


Figure 2

RTA 402 Eudragit SDD 5% and 7.5% HPMC Formulations vs RTA 402 Crystalline (Form A)
Blood Exposure in Cynomolgus Monkeys Following A Single Oral,
Capsule Administration (n=8)
1183-057 MPI (RTA 402-P-0904)





Drug polymorphism and dosage form design: a practical perspective

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Abstract

Formulators are charged with the responsibility to formulate a product which is physically and chemically stable, manufacturable, and bioavailable. Most drugs exhibit structural polymorphism, and it is preferable to develop the most thermodynamically stable polymorph of the drug to assure reproducible bioavailability of the product over its shelf life under a variety of real-world storage conditions. There are occasional situations in which the development of a metastable crystalline or amorphous form is justified because a medical benefit is achieved. Such situations include those in which a faster dissolution rate or higher concentration are desired, in order to achieve rapid absorption and efficacy, or to achieve acceptable systemic exposure for a low-solubility drug. Another such situation is one in which the drug remains amorphous despite extensive efforts to crystallize it. If there is no particular medical benefit, there is less justification for accepting the risks of intentional development of a metastable crystalline or amorphous form. Whether or not there is medical benefit, the risks associated with development of a metastable form must be mitigated by laboratory work which provides assurance that (a) the largest possible form change will have no substantive effect on product quality or bioavailability, and/or (b) a change will not occur under all reasonable real-world storage conditions, and/or (c) analytical methodology and sampling procedures are in place which assure that a problem will be detected before dosage forms which have compromised quality or bioavailability can reach patients. © 2003 Elsevier B.V. All rights reserved.

Keywords: Amorphism; Dissolution; Polymorphism; Dosage form; Bioavailability; Stability; Mechanical properties

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1. Introduction

The subject of drug polymorphism has received extensive academic and industrial attention since the early pioneering reports of Aguiar and colleagues at Parke-Davis, in which effects of polymorphism on dissolution and bioavailability were highlighted for chloramphenicol palmitate [1,2]. Drug polymorphism has been the subject of hundreds of publications and numerous excellent reviews. For both an overview and an in-depth analysis of this complex field, see the excellent series of reviews in Volume 48 (2001) of *Advanced Drug Delivery Reviews* [4–9], in “Polymorphism in Pharmaceutical Sciences” edited by Brittain [10–19], and in “Solid State Chemistry of Drugs” by Byrn et al. [20]. In addition, two very clear reviews/commentaries from the regulatory perspective have appeared [21,22].

At this point in time, it would be difficult to say anything novel about the effects of polymorphism on physical stability, chemical stability, manufacturability, or oral absorption that has not been reviewed in the references quoted above. In many respects, the 1969 review by Haleblan and McCrone was prescient in its broad coverage of the issues of polymorphism in pharmaceuticals [23]. In this article, we make no effort to review once again the vast literature on drug polymorphism. Furthermore, we do not here discuss theoretical or experimental details of the study of polymorphism. Rather, we attempt to provide a practical perspective on the impact of polymorphism on chemical stability, manufacturability, and bioavailability, with particular attention to a limited number of illustrative cases from our experience and the literature. Such a practical perspective must involve generalizations for which there are occasional exceptions.

2. Why develop multiple polymorphs?

It is generally accepted that, during the course of development of a drug, the lowest energy crystalline polymorph should be identified and chosen for development. This is critically important because the post-approval appearance of a polymorph with lower energy than the marketed polymorph can be catastrophic, as happened with the HIV protease inhibitor ritonavir [24]. For this reason, innovator pharmaceutical companies expend significant resources on this technical issue early in the development of a new drug. When executed carefully, the search for the lowest energy polymorph is arduous and time-consuming because (a) a variety of physical and chemical measurements must be made, and the stability of physical and chemical characteristics must be established in real-time storage models, (b) this search is not trivial, because a metastable polymorph may masquerade as the most stable form, and (c) every compound is different (i.e. the identity and properties of polymorphs are not theoretically predictable at present). The search for drug polymorphs is a complex empirical exercise, although recent advances in automation promise to make this activity somewhat less labor intensive.

There are three exceptions to the dictum that only the most stable polymorph should be developed. The first is extremely rare: the situation in which the lowest energy polymorph is chemically unstable due to the juxtaposition of two reactive groups in adjacent molecules in the crystal lattice. Such a “topochemical” reaction can in principle be avoided by identification of a crystalline polymorph in which the reactive species are no longer spatially close and/or oriented in a manner conducive to reaction. We are unaware of any examples of this phenomenon in

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marketed drugs. The second exception is becoming more common, that is, the case of a drug whose absorption is solubility-limited and thus cannot achieve the systemic exposure required for therapy. In this case, a more soluble form of the drug is desired to deliver the therapeutic dose. The third exception is the situation in which it is desired to increase the dissolution rate of a drug to shorten $T_{\max}$ and/or increase $C_{\max}$ in order to bring quick relief for acute symptoms.

In the authors' opinion, when confronted with low solubility or the desire to decrease $T_{\max}$ or increase $C_{\max}$, it is generally more productive to develop a stabilized amorphous form than a metastable crystalline polymorph. This will be discussed in more detail below.

In each of these three exceptions, a metastable polymorph or amorphous form is developed to provide a medical benefit.

If there is a desire to develop a metastable polymorph or amorphous form for a reason which does not provide a medical benefit, e.g. for manufacturing ease or for some other business reason, then the developer must assure that there is no significant risk to the patient. A rigorous laboratory-based analysis of the risks involved must be undertaken. This is of course also true when there is a medical benefit. In the sections below, we discuss the issues involved in the development of metastable polymorphs and amorphous forms, and their potential practical significance.

3. Chemical stability of polymorphs and amorphous forms

The polymorphs (or pseudopolymorphs) of some drugs have been shown to exhibit different chemical stability. Examples are carbamazepine [25], paroxetine maleate [26], indomethacin [27], methyprednisolone [28], furosemide [29], and enalapril maleate [30]. For example, the photodecay of form II of carbamazepine was 5- and 1.5-fold faster than forms I and III, respectively [25]. In addition to a change in the rate of decay, polymorphism may also affect the mechanism of decay, as observed in the reactivity of different polymorphs of cinnamic acid derivatives [31].

It is generally observed that the more thermodynamically stable polymorph is more chemically stable

than a metastable polymorph. This has generally been attributed to higher crystal packing density of the thermodynamically favored polymorph (i.e., the "density rule"), but recent investigation suggests that other factors, such as optimized orientation of molecules, and H-bonds and non-hydrogen bonds in the crystal lattice play a more important role. Relatively small changes in crystal packing may lead to significant differences in the crystal packing density and chemical reactivity of two polymorphs, as indomethacin polymorphs [27]. Indomethacin can exist as the metastable α -form and thermodynamically favored γ -form. As an exception to the density rule, the density of metastable α -form (1.42 g/mL) is higher than that of the γ -form (1.37 g/mL), suggesting tighter packing of the less stable polymorph. Although the metastable α -form has higher density, the α -form rapidly reacts with ammonia vapor while the γ -form is inert to ammonia. The lack in correlation between higher packing density and lower reactivity of the indomethacin polymorphs is due to the differences in crystal packing/hydrogen bonding. Higher density of the α -form is due to the presence of one extra H-bond in the crystal lattice. The differences in H-bonding and the crystal packing (two centrosymmetric carboxylic groups in α -form vs. three asymmetric molecules in γ -form) leads to a layer motif in the α -form that exposes the reactive carboxylic acid group to the crystal face, while in the γ -form, H-bonded carboxylic acid groups are buried in a hydrophobic cage. Easy accessibility of the reactive carboxylic acid groups in the α -form combined with the weak H-bond of one carboxylic acid group leads to higher reactivity of the α -form [27].

The intrinsic difference in chemical stability between two polymorphs, e.g. α - and γ -indomethacin, cannot be overcome, but a less chemically stable polymorph can often be formulated in a way which results in acceptable shelf-life.

In comparison to crystalline polymorphs, the amorphous form of a drug is generally expected to be less chemically stable due to the lack of a three dimensional crystalline lattice, higher free volume and greater molecular mobility. The chemical stability of amorphous systems has been discussed in detail elsewhere [20,32–35]. As early as 1965, amorphous penicillin G was shown to be less stable than the crystalline sodium and potassium salts [36]. Physical

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change of amorphous molecules from a glassy state (at $T < T_g$) to a more mobile supercooled liquid state (at $T > T_g$) may further decrease chemical stability. For example, Asn-hexapeptide was found to be 10–100-fold more stable in the glassy state compared to its supercooled liquid state [37,38]. In addition to higher reactivity, the mechanism of degradation may be different in crystalline versus disordered materials. For example, methyl transfer was the major reaction pathway in unmilled crystalline tetraglycine methyl ester (TGME), while polycondensation was the major reaction pathway in milled TGME [39]. This change in mechanism from methyl transfer to polycondensation upon milling may be due to the creation of a disordered state with higher free volume where molecules can undergo the much higher change in orientation that is needed for the polycondensation reaction [39].

It should be pointed out that a major portion of any formulation effort is the choice of excipients and processes which minimize the chemical instability of the drug. If a metastable polymorph (or amorphous form) is less chemically stable than the lowest energy form of the drug, then in many cases it will be possible to maximize the chemical stability of this metastable form through judicious formulation decisions [40–45]. Thus reduced chemical stability of a metastable crystalline or amorphous drug form does not necessarily preclude its development as a product.

For a more in-depth review of chemical stability and drug physical state, see Byrn et al. [9,20].

4. Mechanical properties of polymorphs and amorphous drug forms

Polymorphism can affect the mechanical properties of drug particles, and thus may impact the manufacturability and physical attributes of tablets. For example, polymorphs of metoprolol tartrate [46], paracetamol [47–50], sulfamerazine [51], phenobarbitone [52], carbamazepine [53,54], phenylbutazone [55] and other drugs have been shown to exhibit different mechanical properties. A common effect of polymorphism is alteration of powder flow due to the difference in particle morphology of two polymorphs. Polymorphs with needle- or rod-shaped particles may have poor flow compared to polymorphs with low

aspect ratio, e.g. cubic habit or irregular spheres. The effect of polymorphism on other mechanical properties, such as hardness, yield pressure, elasticity, compressibility and bonding strength is more complex.

A simple general rule, although semi-empirical, proposed more than 20 years ago by Summers et al. can be used to predict the effect of crystal packing of polymorphs on their compressibility and bonding strength [55,56]. The more stable polymorph, due to its higher packing density, is expected to form stronger interparticle bonds but is harder to deform [46,55,56]. Since an increase in the bonding surface area resulting from deformation of particles may have higher impact on tablet strength than interparticle bond strength, the more stable of two polymorphs may provide weaker tablets. The mechanical properties of two enantiotropic polymorphs of metoprolol tartrate, metastable form I and the more stable form II (at room temperature), are consistent with this rule [46]. The porosity of pure drug tablets and yield pressure for form I were lower than for form II, suggesting that the less dense metastable form I may have less strength in the crystal lattice and be easier to deform. Form I also had higher elastic recovery, probably due to higher elasticity of form I and/or lower porosity of the tablets. As predicted, the tablets of the metastable form I were stronger at low pressures than those of form II, probably due to the higher compressibility of form I.

Factors other than those accounted for by the general rule proposed by Summers et al. may also affect the mechanical properties of two polymorphs. For example, the presence of slip planes in form I of sulfamerazine was found to be the reason for its higher plasticity than form II, the more stable form at room temperature [51]. This higher plasticity results in greater compressibility and tabletability. The authors of this study generalized this observation and suggested that crystals with slip planes would be expected to have superior tableting performance [51]. Recently, a fundamental atom–atom potential model simulation was used to predict a few mechanical properties of sulfathiazole and carbamazepine polymorphs [53]. More fundamental research in this area will improve our ability to predict the effect of polymorphism on mechanical properties.

For amorphous drug forms, mechanical properties may be different from those of crystalline drug due to

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the absence of long range packing. The mechanical attributes of amorphous forms are less well understood than those of crystalline polymorphs. The lack of information on mechanical properties of amorphous drugs may be due to the physical and chemical instability of these forms, leading to reluctance in developing an amorphous form for a commercial drug product. Thus, an evaluation of mechanical properties of amorphous drugs is not routinely investigated in the pharmaceutical industry. One report comparing the mechanical properties of crystalline and amorphous forms of a model drug was published last year [57]. Compacts of amorphous material had higher brittleness and elasticity, and lower ductility than compacts prepared with the crystalline form.

Differences in the mechanical properties of two polymorphs or amorphous versus crystalline forms may or may not affect the manufacturability and physical attributes of tablets. For example, in the case of metoprolol tartrate, the differences in the mechanical properties of two polymorphs did not affect the bonding properties of tablets with relatively high drug loading [46]. The extent of the difference in the mechanical properties of two polymorphs, the drug loading, the robustness of each manufacturing step and the absolute value of the mechanical property undergoing change may be important parameters to consider while assessing the impact of polymorphism on manufacturability and physical attributes of tablets.

In some cases the favorable mechanical properties of one polymorph, even a metastable one, may be used to develop a more desirable process to manufacture tablets. For example, direct compression may be used to manufacture tablets with the more compressible orthorhombic form II of paracetamol instead of using more resource intensive granulation processes for monoclinic form I [47,50]. However, development of a metastable form for processing advantage should only be undertaken for drugs for which a very complete understanding exists with respect to form-dependent chemical stability, physical stability, and most importantly, bioavailability. This will typically be the case only for very old, highly studied, drugs.

As discussed above for chemical stability, manufacturability deficits of a particular polymorph may be overcome through judicious selection of excipients and processes. If a stable polymorph has problematic

mechanical properties, this certainly does not preclude its development. It is much more preferable to use excipients and processing to overcome the mechanical deficits of a stable polymorph than to develop an unstable polymorph because of its better mechanical properties.

For a review of the effects of processing (e.g. tableting) on drug form, see Morris et al. [8] and Brittain and Fiese [17]. For a discussion of the use of excipients to compensate for the physical properties of drugs in formulations, see Amidon [58].

5. Bioavailability of polymorphs

There are many reports of polymorph-dependent bioavailability and/or absorption rate, with much of this work done in animals. See for example animal studies of chloramphenicol palmitate [59], phenylbutazone [60], amobarbital [61], cimetidine [62], 6-mercaptopurine [63], and chlortetracycline [64]. For the purpose of the present analysis, we consider only human studies in detail.

5.1. *Effects of polymorphism on dissolution and oral drug absorption in humans*

Among the best known cases involving human dosing are those of chloramphenicol palmitate, mefenamic acid, oxytetracycline, and carbamazepine. These observations are quite old, having been reported in the 1950s and 1960s. For example, Aguiar et al. [1] demonstrated that absorption of chloramphenicol palmitate polymorph B was significantly greater than absorption of polymorph A in humans. Peak chloramphenicol serum levels were linearly proportional to the percentage of Form B in Form A/Form B mixtures. Chloramphenicol palmitate is a prodrug of chloramphenicol, which was prepared to provide a tasteless derivative [65]. Glazko et al. [66] reported that chloramphenicol palmitate must be hydrolyzed by intestinal esterases before the drug could be absorbed. Aguiar and colleagues demonstrated that in vitro hydrolysis of this prodrug by pancreatin was polymorph dependent, with significant hydrolysis of polymorph B and little hydrolysis of polymorph A. Aguiar and Zelmer [2] demonstrated that Form B dissolves faster than Form A, and has a much higher

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solubility. This solubility difference probably results in the difference in ester hydrolysis rates, and ultimately the difference in oral absorption.

Aguilar and Zelmer [2] also reported on human absorption of two polymorphs of mefenamic acid. In this case, the two polymorphs gave almost identical blood levels. Aguilar and Zelmer calculated a free energy difference (ΔG_T) of -251 cal/mol between the two mefenamic acid polymorphs, where

$$\Delta G_T = RT \ln (\text{Solubility A/Solubility B})$$

In a similar manner, they calculated a free energy difference of -774 cal/mol between polymorphs A and B of chloramphenicol palmitate. These authors pointed out the correlation between the free energy difference and the observation of a polymorph-derived bioavailability difference (seen for chloramphenicol palmitate but not for mefenamic acid). However, the situation is clearly complicated by the issue of hydrolysis of the palmitate moiety in the lumen for chloramphenicol palmitate.

Brice and Hammer [67] reported in 1969 that oral dosing of 16 lots of oxytetracycline capsules from 13 suppliers gave drug blood levels which were lower than the innovator product. Seven of the lots gave oxytetracycline blood levels which were lower than the generally accepted minimum therapeutic level. Blood levels were generally correlated with in vitro dissolution rate. Groves subsequently reported large differences in in vitro dissolution performance of oxytetracycline tablets from various sources [68]. These studies made no attempt to relate dissolution observations to oxytetracycline polymorphism, and the observed differences may have resulted from differing formulations rather than differing polymorphs. Recently, Liebenberg et al. [69] compared six bulk oxytetracycline samples which met USP specifications, and noted that four of these contained one polymorph while the other two contained a different polymorph (form A). Tablets prepared from the form A polymorph dissolved significantly more slowly than the others in 0.1 M HCl. For example, the form A tablets exhibited $\sim 55\%$ dissolution at 30 min, while the others exhibited complete ($\sim 95\%$) dissolution at 30 min.

The drug carbamazepine exhibits polymorphism and product-to-product dissolution and bioavailabili-

ty differences, but a connection between these phenomena has not been directly experimentally demonstrated. Kahela et al. [70] reported that the anhydrous and dihydrate forms of carbamazepine exhibited very similar pharmacokinetics in humans. While the anhydrous form exhibited slower in vitro dissolution than the dihydrate in 0.1 M HCl, inclusion of 0.01% polysorbate 80 in the dissolution medium essentially eliminated this difference. Another study by Jumao-as et al. [71] demonstrated no difference in bioavailability between a generic carbamazepine product and the innovator product. Regardless, carbamazepine therapy with some products has been reported to be problematic [72,73]. Meyer et al. [74] reported on in vitro/in vivo studies of three out of 53 batches of generic carbamazepine tablets which were recalled due to clinical failures and dissolution changes. In vitro dissolution testing, carried out in water containing 1% sodium lauryl sulfate, revealed that two of the batches dissolved more slowly than the innovator product, and one batch dissolved more quickly. While the innovator product gave $\sim 95\%$ dissolution in 90 min in this medium, the slower generic batches gave $\sim 35\%$ and 75% dissolution. In humans, the generic batches gave mean relative AUCs (relative to the innovator) of 60–113%, with the same rank order observed in the in vitro dissolution behavior. It was suggested that moisture uptake during storage and particle size differences may have been involved in the irreproducible behavior of the generic tablets of this practically insoluble drug. It is known that anhydrous carbamazepine converts to the dihydrate quickly, e.g. completely within 1 h, when the anhydrous form is suspended in water [75].

The mechanistic uncertainty in these examples (i.e. whether drug physical form was involved in the observed dissolution or bioavailability differences) results from the lack of spectroscopic data which can identify the drug polymorph in a complex dosage form. Modern techniques such as ss-NMR and NIR can identify polymorphs in dosage forms (within limits), and should facilitate increased mechanistic understanding in future studies.

It is clear that for some drugs, there will be polymorph-dependent bioavailability. For a larger group, there will be polymorph-dependent absorption rate, reflected in in vivo $C_{\max}$. For some pairs of polymorphs, there will be pharmacokinetic bioequi-

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valence. As described above, in 1969 Aguiar and Zelmer proposed that polymorphs with a large free energy difference between them are likely to differ in pharmacokinetic behavior. This simply reflects a difference in solubility. In addition, polymorphs may exhibit different dissolution rates because of their different crystal habits, and this may also contribute to in vivo absorption rate differences.

For an excellent in-depth review of the relationships between polymorphism and solubility and dissolution rate, see Brittain and Grant [16].

5.2. The role of dose in bioavailability of high energy polymorphs

A significant solubility difference between two polymorphs is likely to result in a difference in oral absorption rate, reflected in a difference in $C_{\max}$. Differences in AUC, or oral bioavailability, will occur less often, and will depend upon the same underlying principles which govern the bioavailability differences between two unrelated drugs. Drug absorption may be modeled in a variety of ways [76,77]. A simple context in which to discuss this issue is provided by the concept of the maximum absorbable dose (MAD) [3,78]. The MAD is a conceptual tool which represents the quantity of drug which could be absorbed if the small intestine could be saturated with drug for 4.5 h (270 min), the average small intestinal transit time.

$$\text{MAD} = S \times K_a \times \text{SIWV} \times \text{SITT}$$

S , solubility (mg/ml) at pH 6.5; K_a , transintestinal absorption rate constant (min^{-1}); SIWV, small intestinal water volume (ml); SITT, small intestinal transit time (min).

The solubility at pH 6.5 reflects the solubility in the small intestine. K_a is determined in a rat intestinal perfusion experiment. In our laboratories, it has been observed that the human K_a is 1.4 times the rat K_a [79]. SIWV is the amount of water available for dissolution, generally accepted to be ~ 250 ml. While SIWV and SITT are approximations, moderate variations in these parameters do not significantly affect this analysis. The resulting MAD is in mg. This analysis ignores first pass intestinal and hepatic metabolism, which can be saturated, thus affecting bioavailability.

Table 1
MAD

Rat K_a (min^{-1}) [Human K_a]	Solubility (mg/ml)	MAD (mg) (Human)
0.003 [0.004]	0.01	2.7
0.003 [0.004]	0.02	5.4
0.003 [0.004]	0.03	8.1
0.03 [0.04]	0.01	27
0.03 [0.04]	0.02	54
0.03 [0.04]	0.03	81

If the intent is to increase bioavailability, it can be readily seen that increasing drug solubility will result in increased MAD (Table 1). In general, the range of solubility differences between polymorphs is typically 2–3-fold, due to the relatively small difference in free energy between polymorphs. Thus a higher energy polymorph with a solubility which is $3 \times$ that of the lowest energy polymorph may give a systemic exposure which is $3 \times$ that given by the low energy polymorph. As shown in Table 1, for a low human K_a of 0.004 min^{-1} , and a solubility of 0.01 mg/ml , a 3-fold increase in solubility only results in a MAD of 8.1 mg, which would be inadequate if the desired absorbed dose were, say, 50 mg. If bioavailability were practically governed in this way, there would not be much opportunity to increase the bioavailability of low-solubility drugs by developing a high energy polymorph or amorphous form.

In fact, equilibrium solubility may not be very relevant for oral absorption enhancement if polymorphs (or pseudopolymorphs) are physically unstable in the aqueous environment. Instead, intrinsic dissolution rate (IDR) and kinetic solubility over 4–6 h may be more relevant parameters to consider while studying the oral absorption of polymorphs. Form changes may sometimes occur during IDR and kinetic solubility measurements, but these changes are occurring on a timescale relevant for oral absorption, i.e. the small intestinal transit time. The kinetic solubility of a metastable polymorph over 4–6 h is often higher than its equilibrium solubility. The rank order of the IDR of polymorphs has been found to correlate well with the rank order for oral absorption due to the faster rate of dissolution of the less stable polymorph, leading to higher concentration of drug in solution available for absorption. Generally, this may

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lead to a higher in vivo $C_{\max}$, but not a higher AUC, unless the drug is present in suspension throughout its small intestinal transit time (i.e. the dose is substantially greater than the MAD calculated for the thermodynamically stable polymorph). In some circumstances, the IDR and the achievable metastable supersaturation may temporarily provide a maximum drug concentration in the intestinal lumen which is in excess of the equilibrium solubility of the high energy polymorph. If the drug does not rapidly precipitate in the GI lumen, then the achievable MAD can conceivably be very large.

Although IDR may be a good single parameter to describe relative dissolution rates of two polymorphs, this does not take into account other factors that may govern oral absorption, namely, rate of conversion of one polymorph to another less soluble polymorph in the GI lumen, and the resulting precipitation of drug in the GI fluid. It is generally not possible to theoretically predict the degree of supersaturation of drug from a metastable polymorph or amorphous form, or the kinetics of physical conversion of one polymorph to another. However, these processes may be quantified by comparing the extent of supersaturation in model GI fluid according to Eq. (1), and more importantly Eq. (2):

Supersaturated concentration ratio (SCR)

$$= C_{\max, \text{ form 1}} / C_{\max, \text{ form 2}} \quad (1)$$

Supersaturated AUC ratio (SAR)

$$= \text{AUC}_{\text{form 1}} / \text{AUC}_{\text{form 2}} \quad (2)$$

where $C_{\max, \text{ form 1}}$ and $C_{\max, \text{ form 2}}$ are the in vitro maximum concentrations of drug in solution from forms 1 and 2, respectively; and $\text{AUC}_{\text{form 1}}$ and $\text{AUC}_{\text{form 2}}$ are the areas under the in vitro drug concentration versus time curve over, say, 6 h, for forms 1 and 2, respectively. If a high dose (in substantial excess of the MAD for the stable polymorph) is dosed, and supersaturation is maintained for a long time, e.g. 6 h, while drug is absorbed, then the potential exists to achieve absorption of an amount of drug much higher than the MAD for the stable polymorph.

The greatest effect of dissolution rate and supersaturation of drug from a polymorph or amorphous

form is expected for compounds with high permeability and low solubility relative to dose (i.e. BCS class II compounds, where the administered dose will remain as a suspension for most of the absorption period). For solutes where the dose is expected to be very soluble in the GI fluid (i.e. BCS class I and III compounds) there may be no, or minimal differences in the AUC of polymorphs because solubility is not expected to be rate limiting in oral absorption.

5.3. Potential effects of physical instability of a metastable polymorph on oral absorption

Developing a bioequivalent product with a metastable form may not be easy, but in some cases it may be possible using formulation methods to achieve a bioequivalent AUC. It may be trickier, but possible, to blunt the higher pharmacokinetic $C_{\max}$ which results from the higher dissolution rate of the metastable form. Thus, it may be possible to develop a formulation with a metastable drug form which is bioequivalent to the innovator formulation containing the thermodynamically most stable form. For some drugs, there is a potential danger that bioavailability could be lost if the metastable form converts to the more stable form during the shelf-life of the product. This is illustrated in Table 2. A metastable drug form may be formulated in a product (e.g. tablet) which has the same dissolution rate (Y) as a formulation of the stable drug form. Of course the metastable drug product will have to be formulated in a way which slows the drug dissolution rate. If the metastable form converts to the stable form in the product on storage, then the dissolution rate may decrease and in vivo performance may be compromised. This compromised in vivo performance may involve increased pharmacokinetic

Table 2
Potential performance changes on storage of a dosage form containing a metastable drug form

Drug form in formulation	IDR	Dissolution rate in formulated product	Dissolution rate in product after storage if metastable form converts to stable form
Metastable	$X + \Delta X$	Y	$Y - \Delta Y$
Stable	X	Y	Y

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variability and, more extremely, decreased $C_{\max}$ and bioavailability.

As an example, phenylbutazone Form C exhibits a dissolution rate and solubility which are $1.5 \times$ and $1.2 \times$ that of Form A, respectively [80]. On storage at 40 °C for 12 months, Form C was converted to 60% Form A. As another example, various marketed tablet formulations of glibenclamide have been shown to exhibit differing in vitro dissolution [81]. Glibenclamide exhibits forms which differ greater than 10-fold in solubility in simulated gastric fluid [82]. However, for glibenclamide the connection between product-to-product variability and polymorphism has not been directly demonstrated, but provides a possible explanation.

6. Dosage form decision

6.1. Metastable crystalline polymorph versus amorphous form

As discussed above, metastable crystalline polymorphs and amorphous forms may be less chemically stable and potentially possess different (in some cases less desirable) mechanical properties than the related stable crystalline form. These potential problems can in theory be solved by judicious choice of excipients and appropriate formulation strategies. In addition to chemical instability and mechanical properties, physical stability of the drug during product shelf life is of paramount importance in developing a drug product. A change in physical form can not only affect chemical stability and mechanical attributes of tablets, but much more importantly can compromise the oral absorption of a drug via a change in solubility.

Physical stabilization of intrinsically physically unstable crystalline polymorphs is a challenge because, by definition, the use of additives for improvement of physical stability involves a two phase system (polymorph and stabilizer) where the drug molecules are not in intimate contact with the stabilizer. Furthermore, physical conversion can be relatively precipitous, and exceptional care must be taken to design stability studies which cover all reasonable real-world conditions which such a formulation may encounter (e.g. temperature cycling). There is a need for increased understanding of stabilization of metastable

crystalline forms, and research in this area is sorely needed if practical solutions are to be found.

Amorphous forms are of course also physically unstable. For an introduction to the literature and general concepts on the physical stability of amorphous forms see Yu [5], Yoshioka et al. [83], and Crowley and Zografi [84]. Physical stabilization of amorphous forms is possible in some situations by generating intimate contact between the amorphous drug and the stabilizer by creating a drug/stabilizer dispersion [85–88]. The use of such dispersions, particularly with polymers, to intentionally enhance drug solubility has been known for many years [89,90], and practical formulations which achieve facile low-solubility drug dissolution and supersaturation have recently been described [91,92]. The identification of pharmaceutically acceptable stabilizers and processes which can inhibit solid state crystallization for a reasonable shelf-life is also a recent development [86].

While stabilized amorphous forms can sometimes be developed for intentional bioavailability improvement, the use of such forms to provide a dosage form which is bioequivalent to the stable drug crystalline form would be difficult, but perhaps possible in certain situations.

7. Solvates and hydrates

In general, the analysis provided above for the behavior of polymorphs also applies to metastable solvates and hydrates. For example, the dissolution rate and solubility of a drug can differ significantly for different solvates. Glibenclamide has been isolated as pentanol and toluene solvates, and these solvates exhibit higher solubility and dissolution rate than two non-solvated polymorphs [93]. In formulation of solvates (other than hydrates), the formulator must be careful to address the toxicity of the associated solvent, and carefully evaluate interactions of the drug and mobile solvent molecules with excipients on storage, which may result in compromised performance.

Similar to polymorphs in general, the physical stability of hydrates and anhydrous forms may depend upon the relative humidity and/or temperature of the environment, and the most stable form may switch as

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the humidity/temperature is varied. Anhydrous to hydrate transitions can occur during dissolution at the drug/medium interface and can affect dissolution rate and perhaps bioavailability. Discussion of these issues is beyond the intended scope of this review.

Pharmaceutical solvates and hydrates have been reviewed by Morris [13], and hydrates have been reviewed by Khankari and Grant [94].

8. Conclusions

In principle, any polymorph or hydrate/solvate or amorphous form of a drug can be appropriately formulated. In practice, for some drugs constraints may be encountered. In general, the following conclusions are drawn from the literature and the experience of the authors:

1. It is always advisable to identify the lowest energy crystalline polymorph of a drug candidate during development, and to develop this form. While this form may not be the most processable form available, processing deficits can almost always be overcome with judicious choice of excipients and formulation processes. The lowest energy polymorph is almost always the most chemically stable form, and will not convert to another polymorph during storage as drug product. Of course, care must be taken to avoid conversion during processing to a physically metastable, perhaps chemically unstable, form.
2. Metastable crystalline polymorphs may be less chemically stable than the most physically stable crystalline form. Likewise, amorphous drug forms will generally be less chemically stable than the most physically stable form. It is often possible to improve chemical stability of such forms through judicious choice of excipients and formulation processes.
3. If a developer is precluded from developing the lowest energy drug form, for medical benefit or otherwise, it is preferable to develop a stabilized amorphous form, e.g. as a dispersion. Development of a metastable crystalline or amorphous form as a standard physical mixture or granulation with excipients is less preferable, because it is difficult to guarantee that such a formulation will

resist form changes on storage. If the metastable form converts to the stable less soluble form in the dosage form on storage, then in vivo C_{max} will almost certainly decrease, and in vivo AUC may also decrease depending upon where the drug lies in dose–solubility–permeability space. However, there will be occasional exceptions in which an unstabilized amorphous or metastable crystalline polymorph will be physically stable over the shelf-life of a formulation.

In the end, the manufacturer, whether innovator or generic, must guarantee the quality and bioavailability of the dosage form. It is highly desirable that the drug physical form not change over the storage life of the drug product. If the physical form does change, or if it could change, then the manufacturer must provide assurance (a) that the largest possible change would have no substantive effect on product quality or bioavailability, and/or (b) that extensive scientific study of the formulation guarantees that a change will not occur under all reasonable real-world storage conditions, and/or (c) that analytical methodology and sampling procedures are in place which guarantee that a problem will be detected before dosage forms which have compromised quality or bioavailability can reach patients.

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