

As. 135.767

ANEXOS = 65

62.

Señor

**DIRECTOR DIVISIÓN DE NUEVAS CREACIONES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO**

E.

S.

D.

REF: Expediente No. **16 050.099**– solicitud de patente de invención a favor de **AGIOS PHARMACEUTICALS, INC.** Título: **COMPUESTOS TERAPÉUTICAMENTE ACTIVOS Y SUS MÉTODOS DE USO.**

RESPUESTA A EXAMEN DE FORMA

JUAN PABLO CADENA SARMIENTO, portador de la tarjeta profesional No. 57.492 del C.S.J., obrando como apoderado de la sociedad solicitante me permito dar respuesta al Oficio No. 7020 notificado por fijación en lista el 21 de Junio de 2016.

En respuesta a las observaciones realizadas como resultado del examen de forma, es pertinente mencionar que las reivindicaciones definen de forma clara y razonablemente general la invención, razón por la que respetuosamente disiento de los comentarios del señor Examinador a este respecto. A continuación, sin embargo, se incluyen los argumentos y se mencionan las modificaciones realizadas para superar cada una de las objeciones del Señor Examinador:

Título

De acuerdo con la sugerencia del Señor Examinador, se solicita el cambio del título de la solicitud a “POLIMORFOS DE 2-METIL-1-[(4-[6-(TRIFLUOROMETIL)PIRIDIN-2-IL]-6-[[2-(TRIFLUOROMETIL)PIRIDIN-4-IL]AMINO}-1,3,5-TRIAZIN-2-IL)AMINO]PROPAN-2-OL Y SALES DE LOS MISMOS”.

Poder

Adjunto a este memorial se presenta el documento de poder otorgado por la solicitante.

Excepciones de patentabilidad

Las reivindicaciones 21 a 48 han sido eliminadas del pliego reivindicatorio, por lo que las objeciones dirigidas a éstas no prosperan en el nuevo pliego.

Reivindicaciones

En este punto se debe señalar que los compuestos 1 y 3 han sido reemplazados por “metasulfonato de 2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il]amino]propan-2-ol” y “2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il]amino]propan-2-ol” respectivamente, definiendo así la invención de forma clara y suficiente.

Haciendo referencia ahora a la objeción a las reivindicaciones 1, 3, 5, 7, 9, 11, 13, 15, 17 y 19 por no incluir suficientes picos característicos del patrón de difracción con polvo por rayos X, se debe resaltar que el Señor Examinador no ha provisto evidencia alguna que soporte que los picos identificados en las reivindicaciones no sean suficientes para identificar de forma única cada una de las formas cristalinas de la presente invención. Al respecto, la presente solicitud provee una caracterización estructural detallada de cada uno de los polimorfos reivindicados, como se determina por la calorimetría por barrido diferencial (DSC), los patrones de difracción de rayos X y el análisis gravimétrico térmico (TGA).

En el estado de la técnica existen numerosas referencias que soportan que, cuando se encuentran disponibles caracterizaciones detalladas de cada polimorfo de un compuesto, es posible identificar de forma única cada polimorfo mediante un número limitado de picos en el patrón de difracción de rayos X. De hecho, en algunos casos un único pico es suficiente para identificar de forma única un polimorfo que ha sido previamente caracterizado.

Por ejemplo, el artículo "Characterization of the Distribution, Polymorphism, and Stability of Nimodipine in its Solid Dispersions in Polyethylene Glycol by Micro-Raman Spectroscopy and Powder X-Ray Diffraction" (adjunto) describe como las formas cristalinas I y II de los cristales de nimodipina son cada una identificadas (y por lo tanto diferenciadas entre sí) por un único pico en el patrón de difracción de rayos X (ver página E363, columna 2 párrafo 1). Por otro lado, el artículo "Polymorphism in Drugs and its Significance in Therapeutics" (adjunto) enseña varios antibióticos cuyas formas polimórficas son distinguidas entre sí mediante tres picos de su patrón de difracción de rayos X; dentro del vasto número de antibióticos se encuentran los siguientes: nistatina (tres polimorfos cristalinos), Cefradina (cuatro polimorfos cristalinos), indometacina (dos polimorfos cristalinos), fenilbutazona (tres polimorfos cristalinos) y captopril (dos polimorfos cristalinos). Similarmente, el artículo "Crystal Polymorphism in a Carbamazepine Derivative: Oxcarbazepine" (adjunto) enseña la identificación de tres formas polimórficas cristalinas de un compuesto, carbamazepina, usando entre tres y cinco picos en el patrón de difracción de rayos X para distinguir las formas entre sí.

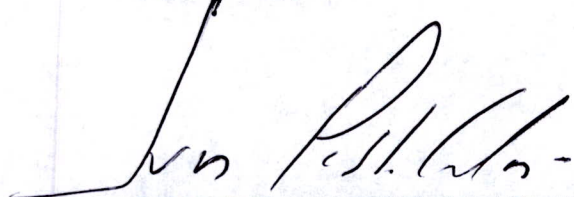
Así, teniendo en cuenta las enseñanzas provistas en la descripción de la solicitud referentes a la caracterización detallada de las formas polimórficas reivindicadas, y el conocimiento de la persona versada en la materia, los picos en el patrón de difracción de rayos X especificados en las reivindicaciones son suficientes para identificar de forma única cada una de las formas polimórficas.

Sin perjuicio de lo anterior, se han agregado picos adicionales en cada una de las reivindicaciones con el fin de favorecer el trámite de la solicitud.

Por último, las reivindicaciones 2, 4, 6, 8, 10, 12, 14, 16, 18 y 20 han sido eliminadas del pliego reivindicatorio, por lo que las objeciones dirigidas a éstas no prosperan en el nuevo pliego.

En vista de lo anteriormente expuesto, respetuosamente solicito el retiro de las objeciones de la presente solicitud, ya que en opinión del solicitante cumple con todos los requisitos establecidos en la Decisión 486.

Atentamente,



JUAN PABLO CADENA SARMIENTO

T.P. No. 57.492 del C.S.J.

C.C. 80.410.337 de Usaquén

Calle 70 A No. 4 - 41

REIVINDICACIONES

1. Una forma cristalina aislada del compuesto 2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il)amino]propan-2-ol, en donde la forma cristalina aislada es la Forma 16, caracterizada por un patrón de difracción con polvo por rayos X que tiene picos en los ángulos 2Θ de 6.8, 10.1, 10.6, 13.6, 14.2, 17.2, 18.4, 19.2, y $23.5^\circ \pm 0.2^\circ$.
2. Una forma cristalina aislada del compuesto 2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il)amino]propan-2-ol, en donde la forma cristalina aislada es la Forma 1, caracterizada por un patrón de difracción con polvo por rayos X que tiene picos en los ángulos 2Θ de 6.7, 8.9, 9.1, 13.0, 16.4, 18.9, 21.4, 23.8, y $28.1^\circ \pm 0.2^\circ$.
3. Una forma cristalina aislada del compuesto 2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il)amino]propan-2-ol, en donde la forma cristalina aislada es la Forma 2, caracterizada por un patrón de difracción con polvo por rayos X que tiene picos en los ángulos 2Θ de 8.4, 12.7, 16.9, 17.1, 17.7, 19.2, 23.0, 23.3, y $24.2^\circ \pm 0.2^\circ$.
4. Una forma cristalina aislada del compuesto metasulfonato de 2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il)amino]propan-2-ol, en donde la forma cristalina aislada es la Forma 3, caracterizada por un patrón de difracción con polvo por rayos X que tiene picos en los ángulos 2Θ de 7.5, 9.0, 9.3, 14.5, 15.2, 18.0, 18.8, 19.9, 21.3, y $24.8^\circ \pm 0.2^\circ$.
5. Una forma cristalina aislada del compuesto metasulfonato de 2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il)amino]propan-2-ol, en donde la forma cristalina aislada es la Forma 7, caracterizada por un patrón de difracción con polvo por rayos X que tiene picos en ángulos 2Θ de 9.7, 14.1, 18.6, 19.1, 20.2, 21.8, 23.5, 25.7, y $28.9^\circ \pm 0.2^\circ$.
6. Una forma cristalina aislada del compuesto metasulfonato de 2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il)amino]propan-2-ol, en donde la forma cristalina aislada es la Forma 8, caracterizada por un patrón de difracción con polvo por rayos X que tiene picos en los ángulos 2Θ de 9.0, 9.2, 14.1, 16.8, 19.9, 21.9, 22.1, 24.2, y $24.6^\circ \pm 0.2^\circ$.
7. Una forma cristalina aislada del compuesto metasulfonato de 2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il)amino]propan-2-ol, en donde la forma cristalina aislada es la Forma 9, caracterizada por un patrón de difracción con polvo por rayos X que tiene picos en los ángulos 2Θ de 6.5, 10.7, 17.7, 18.4, 19.0, 19.6, 20.1, 21.6, y $29.9^\circ \pm 0.2^\circ$.

8. Una forma cristalina aislada del compuesto 2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il)amino]propan-2-ol, en donde la forma cristalina aislada es la Forma 17, caracterizada por un patrón de difracción con polvo por rayos X que tiene picos en ángulos 2Θ de 7.2, 10.1, 11.5, 13.6, 18.5, 19.3, 20.3, 21.9, y $23.5^\circ \pm 0.2^\circ$.
9. Una forma cristalina aislada del compuesto 2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il)amino]propan-2-ol, en donde la forma cristalina aislada es la Forma 18, caracterizada por un patrón de difracción con polvo por rayos X que tiene picos en los ángulos 2Θ de 6.4, 8.4, 9.8, 16.1, 16.9, 17.8, 19.7, 21.1, y $26.1^\circ \pm 0.2^\circ$.
10. Una forma cristalina aislada del compuesto 2-metil-1-[(4-[6-(trifluorometil)piridin-2-il]-6-[[2-(trifluorometil)piridin-4-il]amino]-1,3,5-triazin-2-il)amino]propan-2-ol, en donde la forma cristalina aislada es la Forma 19, caracterizada por un patrón de difracción con polvo por rayos X que tiene picos en ángulos 2Θ de 8.1, 11.4, 14.1, 15.2, 16.4, 17.3, 20.5, y $24.1^\circ \pm 0.2^\circ$.

Brigard & Castro

BOGOTÁ - COLOMBIA

12827-843-026

PODER

Yo (nosotros), **AGIOS PHARMACEUTICALS, INC.**

domiciliado(s) en 88 Sidney Street, Cambridge, MA 02139, US.

For national phase application PCT/US2014/049469 entitled THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE

Nombro(amos) a: **JUAN PABLO CADENA SARMIENTO** y/o **MARTIN E. TORRES CARDOZO**, de Bogotá, Colombia, apoderados verdaderos y legales con poder especial, amplio y suficiente, para recabar de las oficinas y autoridades colombianas administrativas, judiciales y de policía, en cualquier instancia o recurso, la obtención de patentes de invención, registros de marcas, diseños industriales, modelos de utilidad, depósitos de nombres comerciales y enseñas, lemas comerciales, nombres comerciales, registros de derechos de autor y contratos sobre los mismos, obtención de certificados de obtentor de variedades vegetales, así como sus prórrogas, renovaciones, anotaciones de traspaso, cambios de nombre y/o domicilio y/o dirección; elaborar, tramitar y registrar contratos de licencia y licencias de cualesquiera clases; intervenir como demandante o como demandado en cualquier instancia y recurso ante cualquier juez, corporación, funcionario público o autoridad en acciones judiciales, administrativas y de policía tales como: oposiciones al registro de marcas; acciones de caducidad, cancelación y nulidad; acciones reivindicatorias, acciones derivadas de la obtención o explotación de licencias; acciones de competencia desleal; demandas penales; acciones de indemnización de perjuicios, reclamos y observaciones a solicitudes de patentes, diseños industriales, modelos de utilidad, en el ejercicio de medidas cautelares y en otras acciones o medidas similares.

A este efecto, lo(s) faculto(amos) para elevar solicitudes, formular descripciones, enmiendas, protestas, declaraciones, oposiciones, cancelaciones, nulidades, apelaciones y reclamos; pagar impuestos, cuotas y gravámenes prescritos por la ley; renunciar o desistir a derechos concedidos o en curso de concesión; recibir toda clase de documentos y valores, dando el descargo respectivo, y, en general, para dar ante dichas autoridades todos los pasos necesarios al efecto indicado y tomar todas las medidas que creyeran conducentes al resguardo de mis (nuestros) intereses, con todas las demás facultades legales necesarias.

Este poder comprende la facultad de ratificar o confirmar en mi (nuestro) nombre lo actuado así como la facultad de desistir, transigir, comprometer, y la de sustituirlo en todo o en parte y revocar sustituciones. Asimismo nombro(amos) a: **JUAN PABLO CADENA SARMIENTO** y/o **MARTIN E. TORRES CARDOZO**, domiciliado(s) en la calle 70A No. 4-41, mis (nuestros) apoderados en Colombia, con facultades para recibir notificaciones y designar apoderados judiciales o extrajudiciales. Otorgado y firmado en

hoy de de 2016
Firma
Nombre
Cargo

POWER OF ATTORNEY

I (We), **AGIOS PHARMACEUTICALS, INC.**

domiciled in 88 Sidney Street, Cambridge, MA 02139, US.

For national phase application PCT/US2014/049469 entitled THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE

Hereby appoints **JUAN PABLO CADENA SARMIENTO** and/or **MARTIN E. TORRES CARDOZO**, of Bogotá, Colombia, my (our) true and lawful attorneys with special, ample and sufficient power to obtain from the Colombian administrative, judicial and police offices and authorities, in any instance, privileges of patents of invention, registration of trademarks, industrial designs and utility models; registration of commercial names, slogans and emblems, registration of copyright and contracts related thereto, certificates of obtainers of vegetables varieties, as well as extensions, renewals and recordal of assignments and changes of name, domicile and address related to the above; to prepare, prosecute and register licenses of any kind; to act as plaintiff or defendant in any instance or recourse, and before any judge, corporation, public official or authority in judicial, administrative and police actions, such as: oppositions to the registration of trademarks; caducity, cancellation and nullity actions; claim actions derived from the obtaining or exploitation of licenses action of unfair competition; criminal proceedings, action for payment of damages; claims or observations on patent applications, models, industrial designs and utility mode in the exercise of preventive measures and in other actions or similar proceedings.

To that effect they hereby empowered to file applications, formulate descriptions, amendments, declarations, oppositions, cancellations, nullities, and claims; to pay taxes, quotas and assessments prescribed by law, renounce or waive the rights in the process of granting; to receive all documents and valuables, giving the respective receipt, and, in general, to take before said authorities all necessary steps to such effect, and any measure they may deem pertinent to the safeguarding of my (our) interest(s) with all the other necessary legal powers.

This power includes the faculty to ratify or settle and compromise on my (our) behalf, to substitute this power in whole or in part by substitutions. At the same time I (We) **JUAN PABLO CADENA SARMIENTO** and/or **MARTIN E. TORRES CARDOZO**, domiciled in calle 70A No. 4-41, my (our) attorney(s) in fact, with powers to receive notifications and to designate judicial and extrajudicial attorneys.

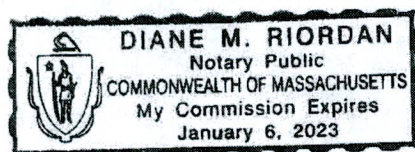
Granted and signed in Cambridge, MA, USA
this 4th day February of 2016

Signature _____
Name Min Wang, Ph.D., J.D.
Title VP of Legal and Secretary

COMMONWEALTH OF MASSACHUSETTS)
) ss.:
COUNTY OF MIDDLESEX)

On this 4th day of February, 2016, before me, the undersigned Notary Public, personally appeared Min Wang, Ph.D., J.D., proved to me through satisfactory evidence of identification, which was a driver's license to be the person whose name is signed on the preceding or attached document in my presence.

WITNESS my hand and notarial seal, this 4th day of February, 2016.



Deane M. Rendon
Notary Public

Apostille

(Convention de La Haye du 5 Octobre 1961)

1. Country: United States of America
This public document
2. has been signed by **Milton A. Tingling**
3. acting in the capacity of **County Clerk**
4. bears the seal/stamp of the **county of New York**

Certified

5. at New York City, New York
6. the 17th day of February 2016
7. by Special Deputy Secretary of State, State of New York
8. No. NYC-536354
9. Seal/Stamp
10. Signature



Whitney A. Clark

Whitney A. Clark
Special Deputy Secretary of State

10

Form 1

State of New York }
County of New York } ss:

No. 185627

I, **Milton Adair Tingling**, Clerk of the County of New York, and Clerk of the Supreme Court in and for said county, the same being a court of record having a seal, **DO HEREBY CERTIFY THAT**

AMY LEWIS



whose name is subscribed to the annexed original instrument has been commissioned and qualified as a NOTARY PUBLIC.....
and has filed his/her original signature in this office and that he/she was at the time of taking such proof or acknowledgment or oath duly authorized by the laws of the State of New York to take the same: that he/she is well acquainted with the handwriting of such public officer or has compared the signature on the certificate of proof or acknowledgment or oath with the original signature filed in his/her office by such public officer and he/she believes that the signature on the original instrument is genuine.

IN WITNESS WHEREOF, I have hereunto set my hand and my official seal this
16th day of February, 2016

Milton Adair Tingling

County Clerk, New York County



20

10

VERIFICATION

STATE OF NEW YORK)
) ss:
COUNTY OF NEW YORK)

I verify that the attached document identified as:

Assignment

is a copy of the document as executed on July 23, 2014 by Liting Guo, on July 25, 2014 by Zhen Tang, Jianming Wang, Yanfeng Zhang and Yan Zhou, to **CRYSTAL PHARMATECH, INC.** of United States and corresponding foreign rights in connection with PCT/CN2013/081170 filed August 9, 2013.

Corinne Zang
Corinne Zang
JONES DAY
International Patent
Applications Supervisor

COUNTY
CLERK

W YORK
COUNTY

Subscribed and sworn to before me this 2nd day of

February, 2016.

Amy Lewis
Notary Public

AMY LEWIS
Notary Public, State of New York
No. 01LE5130070
Qualified in Kings County
Certificate Filed in New York County
Commission Expires Jan. 7, 2020

ASSIGNMENT

For good and valuable consideration, the sufficiency and receipt of which is hereby acknowledged, I, Liting GUO (Citizenship: China), Room 406, Bldg. 27, Xietang lotus 8 area, Suzhou Industrial Park, Suzhou 215123, China; Zhen TANG (Citizenship: China), Room 303, Bldg. 26, JingYing apartment, 01 Qi Yue Street Suzhou Industrial Park, Suzhou 215123, China; Jianming WANG (Citizenship: China), 1503, Bldg. 15, Scholar's Garden, Suzhou Industrial Park, Suzhou 215123, China; Yanfeng ZHANG (Citizenship: China), Room 603, Bldg. 29, JingYing apartment, 01 Qi Yue Street Suzhou Industrial Park, Suzhou 215123, China; Yan ZHOU (Citizenship: China), Room 901, Bldg. 13, JingYing apartment, 01 Qi Yue Street, Suzhou Industrial Park, Suzhou 215123, China, hereby:

Sell, assign and transfer to Crystal PharmaTech, Inc., B4-101, Biobay, 218 Xinghu Street, Suzhou Industrial Park, Suzhou 215125, China, its successors, assigns and legal representatives, all hereinafter referred to as said Assignee, the entire right, title and interest for the United States and all foreign countries, in and to any and all inventions which are disclosed in the International Application for Patent filed with the State Intellectual Property Office of the People's Republic of China as Receiving Office of the World Intellectual Property Organization in accordance with the Patent Cooperation Treaty (PCT) on

Filed: _____
under International Application Serial No. _____

and

entitled THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE.

and in and to said application and all continuing, divisional, continuation-in-part, substitute, renewal, reissue and all other applications for Letters Patent which have been or shall be filed in the United States and all foreign countries on said any and all inventions (including without limitation International Application Serial No. PCT/CN2013/081170, filed August 9, 2013, and entitled CRYSTALLINE FORMS OF THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE); and in and to all original and reissued patents which have been or shall be issued in the United States and all foreign countries on said any and all inventions including the right to apply for patent rights in each foreign country and all rights to priority; and further including any right to sue for past infringement;

Appoint, authorize and request the attorneys appointed in said application to hereafter complete this assignment by inserting in the spaces provided above the filing date and serial number of said application when known;

Agree that said Assignee may apply for and receive Letters Patent for said any and all inventions in its own name; and when requested, without charge to but at the expense of said Assignee, I agree to carry out in good faith the intent and purpose of this assignment, by executing all divisional, continuing, substitute, renewal, reissue, and all other patent applications on said any and all inventions, by executing all rightful oaths, assignments, powers of attorney and other papers, by communicating to said Assignee all facts known to me relating to said any and all

13 12

Attorney Docket No.: C2081-7065WO

inventions and the history thereof, and generally by doing everything reasonably possible which said Assignee shall consider desirable for aiding in securing and maintaining proper patent protection for said any and all inventions and for vesting title to said any and all inventions and all applications for patents and all patents on said inventions, in said Assignee;

Request the Honorable Commissioner of Patents and Trademarks to issue said Letters Patent to said Assignee; and

Covenant with said Assignee that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by me and that full right to convey the same as herein expressed is possessed by me.

Said Assignee does hereby acknowledge and accept the above sale, assignment and transfer of all the rights, title and interest enumerated above, including but not limited to the right to priority and the right to sue for past infringement.

In the event that the execution date, filing date and/or Application No. are not entered or are incorrect at the time Assignor(s) executed this document, Assignor(s) hereby authorize and request an attorney of Assignee to insert the information or make the correction.

AC

24

14

THE ASSIGNOR

23-Jul-2014
Date

Liting Guo
Assignor: Liting GUO

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Yan Zhou

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THE ASSIGNOR

25-Jul-2014
Date

Zhen Tang
Assignor: Zhen TANG

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Address Suzhou, 215123, China

15

THE ASSIGNOR

25-Jul-2014
Date

Wjm
Assignor: Jianming WANG

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Lifang Lu

Witness

Name

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Industrial Park, Suzhou 215123, China

THE ASSIGNOR

25-Jul-2014
Date

Yanfeng
Assignor: Yanfeng ZHANG

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THE ASSIGNOR

25 Jul 2014
Date

Yan Zhou
Assignor: Yan ZHOU

Don Feng

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Lifang Lu

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Suzhou Industrial Park, Suzhou 215123, China

17

Attorney Docket No.: C2081-7065WO

ASSIGNEE: Crystal PharmaTech, Inc.

28 Jul 2014
Date

Alex Chen
Signature

Alex Chen

Printed Name of Signor

CEO

Title/Authority of Signor

Don Feng

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Lifang Lu

Witness Name
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Industrial Park, Suzhou 215123, China

Apostille

(Convention de La Haye du 5 Octobre 1961)

1. Country: United States of America
This public document
2. has been signed by **Milton A. Tingling**
3. acting in the capacity of **County Clerk**
4. bears the seal/stamp of the **county of New York**

Certified

5. at New York City, New York 6. the 17th day of February 2016
7. by Special Deputy Secretary of State, State of New York
8. No. NYC-536349
9. Seal/Stamp 10. Signature



Whitney A. Clark

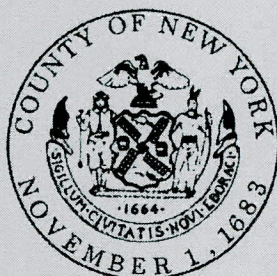
Whitney A. Clark
Special Deputy Secretary of State

State of New York }
County of New York } ss:

No. 185628

I, **Milton Adair Tingling**, Clerk of the County of New York, and Clerk of the Supreme Court in and for said county, the same being a court of record having a seal, **DO HEREBY CERTIFY THAT**

AMY LEWIS

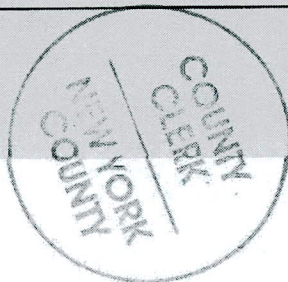


whose name is subscribed to the annexed original instrument has been commissioned and qualified as a NOTARY PUBLIC.....
and has filed his/her original signature in this office and that he/she was at the time of taking such proof or acknowledgment or oath duly authorized by the laws of the State of New York to take the same: that he/she is well acquainted with the handwriting of such public officer or has compared the signature on the certificate of proof or acknowledgment or oath with the original signature filed in his/her office by such public officer and he/she believes that the signature on the original instrument is genuine.

IN WITNESS WHEREOF, I have hereunto set my hand and my official seal this
16th day of February, 2016

Milton Adair Tingling

County Clerk, New York County



NEW YORK
COUNTY
CLERK
7, 2016

20

12827-843-078

VERIFICATION

STATE OF NEW YORK)
) ss:
COUNTY OF NEW YORK)

I verify that the attached document identified as:

Assignment

is a copy of the document as executed on July 28, 2014 by
CRYSTAL PHARMATECH, INC., to **AGIOS PHARMACEUTICALS, INC.** of
United States and corresponding foreign rights in connection
with PCT/CN2013/081170 filed August 9, 2013.

Corinne Zang
Corinne Zang
JONES DAY
International Patent
Applications Supervisor

Subscribed and sworn to before me this 2nd day of
February, 2016.

Amy Lewis
Notary Public



AMY LEWIS
Notary Public, State of New York
No. 01LES100070
Qualified in Kings County
Certification Filed in New York County
Commission Expires Jan. 7, 2020

ASSIGNMENT

For good and valuable consideration, the sufficiency and receipt of which is hereby acknowledged, Crystal PharmaTech, Inc., B4-101, Biobay, 218 Xinghu Street, Suzhou Industrial Park, Suzhou 215125, China, hereby:

Sell, assign and transfer to Agios Pharmaceuticals, Inc., a Delaware corporation having a place of business at 38 Sidney Street, Cambridge, Massachusetts 02139, United States of America, its successors, assigns and legal representatives, all hereinafter referred to as said Assignee, the entire right, title and interest for the United States and all foreign countries, in and to any and all inventions which are disclosed in the International Application for Patent filed with the State Intellectual Property Office of the People's Republic of China as Receiving Office of the World Intellectual Property Organization in accordance with the Patent Cooperation Treaty (PCT) on

Filed: _____
under International Application Serial No. _____
and

entitled THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE,

and in and to said application and all continuing, divisional, continuation-in-part, substitute, renewal, reissue and all other applications for Letters Patent which have been or shall be filed in the United States and all foreign countries on said any and all inventions (including without limitation International Application Serial No. PCT/CN2013/081170, filed August 9, 2013, and entitled CRYSTALLINE FORMS OF THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE); and in and to all original and reissued patents which have been or shall be issued in the United States and all foreign countries on said any and all inventions including the right to apply for patent rights in each foreign country and all rights to priority; and further including any right to sue for past infringement;

Appoint, authorize and request the attorneys appointed in said application to hereafter complete this assignment by inserting in the spaces provided above the filing date and serial number of said application when known;

Agree that said Assignee may apply for and receive Letters Patent for said any and all inventions in its own name; and when requested, without charge to but at the expense of said Assignee, I agree to carry out in good faith the intent and purpose of this assignment, by executing all divisional, continuing, substitute, renewal, reissue, and all other patent applications on said any and all inventions, by executing all rightful oaths, assignments, powers of attorney and other papers, by communicating to said Assignee all facts known to me relating to said any and all inventions and the history thereof, and generally by doing everything reasonably possible which said Assignee shall consider desirable for aiding in securing and maintaining proper patent protection for said any and all inventions and for vesting title to said any and all inventions and all applications for patents and all patents on said inventions, in said Assignee;

22

Attorney Docket No.: C2081-7065WO

Request the Honorable Commissioner of Patents and Trademarks to issue said Letters Patent to said Assignee; and

Covenant with said Assignee that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by me and that full right to convey the same as herein expressed is possessed by me.

Said Assignee does hereby acknowledge and accept the above sale, assignment and transfer of all the rights, title and interest enumerated above, including but not limited to the right to priority and the right to sue for past infringement.

In the event that the execution date, filing date and/or Application No. are not entered or are incorrect at the time Assignor(s) executed this document, Assignor(s) hereby authorize and request an attorney of Assignee to insert the information or make the correction.

HC

27
23

Attorney Docket No.: C2081-7065WO

By: Crystal PharmaTech, Inc.

28-Jul-2014
Date

Alex Chen
Signature

Alex Chen
Printed Name of Signor

CEO
Title/Authority of Signor

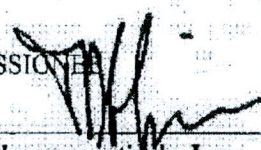
Lifang Lu
Witness

Name Room 1204, Bldg 04, JingYing apartment, 01 QiYue Street, Suzhou
Address Industrial Park, Suzhou 215123, China

Yan zhou
Witness

Name Room 901, Bldg 13, JingYing apartment, 01 QiYue Street, Suzhou Industrial park,
Address Suzhou, 215123, China

THE ASSIGNEE


Agiros Pharmaceuticals, Inc.

Authorized Representative

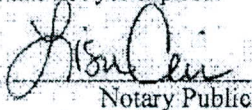
By: **J. Duncan Higgins**
Chief Operating Officer

Title:

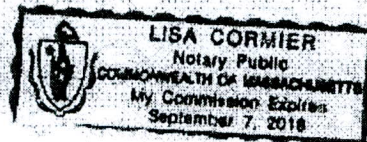
Given and signed in Cambridge, MA this 22nd day of July 2014.

NOTARIAL CERTIFICATE

On this 22nd day of July of 2014, personally appeared before me J. Duncan Higgins, whose signature is authentic and was duly identified. Duly sworn he declared that he is the COO of Agiros Pharmaceuticals, Inc. I further certify that the person appearing before me is authorized to sign this document on behalf of this corporation, which according to the Charter and By Laws is a legally existing and duly organized corporation, as its duration term has not yet expired.


Notary Public

{Notary Seal}



25
25

Apostille

(Convention de La Haye du 5 Octobre 1961)

1. Country: United States of America
This public document
2. has been signed by **Milton A. Tingling**
3. acting in the capacity of **County Clerk**
4. bears the seal/stamp of the **county of New York**

Certified

5. at New York City, New York
6. the 17th day of February 2016
7. by Special Deputy Secretary of State, State of New York
8. No. NYC-536352
9. Seal/Stamp
10. Signature



Whitney A. Clark

Whitney A. Clark
Special Deputy Secretary of State

26

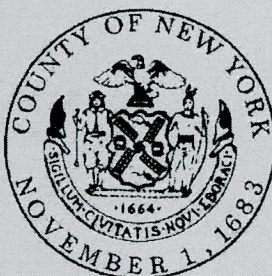
Form 1

State of New York }
County of New York } ss:

No. 185625

I, **Milton Adair Tingling**, Clerk of the County of New York, and Clerk of the Supreme Court in and for said county, the same being a court of record having a seal, **DO HEREBY CERTIFY THAT**

AMY LEWIS

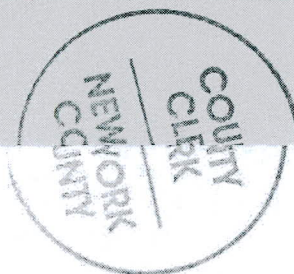


whose name is subscribed to the annexed original instrument has been commissioned and qualified as a NOTARY PUBLIC.....
and has filed his/her original signature in this office and that he/she was at the time of taking such proof or acknowledgment or oath duly authorized by the laws of the State of New York to take the same; that he/she is well acquainted with the handwriting of such public officer or has compared the signature on the certificate of proof or acknowledgment or oath with the original signature filed in his/her office by such public officer and he/she believes that the signature on the original instrument is genuine.

IN WITNESS WHEREOF, I have hereunto set my hand and my official seal this
16th day of February, 2016

Milton Adair Tingling

County Clerk, New York County



120

26

72

VERIFICATION

STATE OF NEW YORK)
) ss:
COUNTY OF NEW YORK)

I verify that the attached document identified as:

Assignment

is a copy of the document as executed on July 28, 2014 by Chong-Hui-Gu and Hua Yang, to **AGIOS PHARMACEUTICALS, INC.** of United States and corresponding foreign rights in connection with PCT/CN2013/081170 filed August 9, 2013.

Corinne Zang
Corinne Zang
JONES DAY
International Patent
Applications Supervisor

Subscribed and sworn to before me this 2nd day of February, 2016.

Amy Lewis
Notary Public

AMY LEWIS
Notary Public, State of New York
No. 01LE6180070
Qualified in Kings County
Certificate Filed in New York County
Commission Expires Jan. 7, 2020

NTY
RK
YORK
UNTY

28

ASSIGNMENT

For good and valuable consideration, the sufficiency and receipt of which is hereby acknowledged, I, Chong-Hui GU (Citizenship: United States of America), 217 Varick Road, Waban, Massachusetts 02468, United States of America; and Hua YANG (Citizenship: United States of America), 48 Conant Street, Acton, Massachusetts 01720, United States of America hereby:

Sell, assign and transfer to Agios Pharmaceuticals, Inc., a Delaware corporation having a place of business at 38 Sidney Street, Cambridge, Massachusetts 02139, United States of America, its successors, assigns and legal representatives, all hereinafter referred to as said Assignee, the entire right, title and interest for the United States and all foreign countries, in and to any and all inventions which are disclosed in the International Application for Patent filed with the State Intellectual Property Office of the People's Republic of China as Receiving Office of the World Intellectual Property Organization in accordance with the Patent Cooperation Treaty (PCT) on

Filed _____

under International Application Serial No. _____

and

entitled THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE,

and in and to said application and all continuing, divisional, continuation-in-part, substitute, renewal, reissue and all other applications for Letters Patent which have been or shall be filed in the United States and all foreign countries on said any and all inventions (including without limitation International Application Serial No. PCT/CN2013/081170, filed August 9, 2013, and entitled CRYSTALLINE FORMS OF THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE); and in and to all original and reissued patents which have been or shall be issued in the United States and all foreign countries on said any and all inventions including the right to apply for patent rights in each foreign country and all rights to priority; and further including any right to sue for past infringement;

Appoint, authorize and request the attorneys appointed in said application to hereafter complete this assignment by inserting in the spaces provided above the filing date and serial number of said application when known;

Agree that said Assignee may apply for and receive Letters Patent for said any and all inventions in its own name; and when requested, without charge to but at the expense of said Assignee, I agree to carry out in good faith the intent and purpose of this assignment, by executing all divisional, continuing, substitute, renewal, reissue, and all other patent applications on said any and all inventions, by executing all rightful oaths, assignments, powers of attorney and other papers, by communicating to said Assignee all facts known to me relating to said any and all inventions and the history thereof, and generally by doing everything reasonably possible which said Assignee shall consider desirable for aiding in securing and maintaining proper patent protection for said any and all inventions and for vesting title to said any and all inventions and all applications for patents and all patents on said inventions, in said Assignee;

Request the Honorable Commissioner of Patents and Trademarks to issue said Letters Patent to said Assignee; and

Covenant with said Assignee that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by me and that full right to convey the same as herein expressed is possessed by me.

Said Assignee does hereby acknowledge and accept the above sale, assignment and transfer of all the rights, title and interest enumerated above, including but not limited to the right to priority and the right to sue for past infringement.

In the event that the execution date, filing date and/or Application No. are not entered or are incorrect at the time Assignor(s) executed this document, Assignor(s) hereby authorize and request an attorney of Assignee to insert the information or make the correction.

30

THE ASSIGNOR

[Signature]

Chong-Hui GU

Given and signed in Cambridge, MA this 28th day of July 2014.

State of Massachusetts

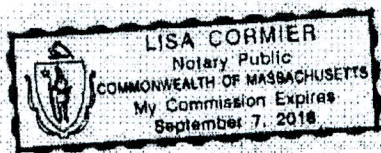
County of Middlesex

NOTARIAL CERTIFICATE

The foregoing instrument was acknowledged before me this 28th day of July, 2014, by Chong-Hui GU. / Personally known or Produced as identification.

[Signature]
Notary Public

{Notary Seal}



31

THE ASSIGNOR

[Signature]

Hua YANG

Given and signed in Cambridge, MA this 22nd day of July 2014.

State of Massachusetts

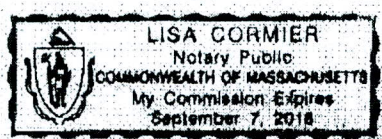
County of Middlesex

NOTARIAL CERTIFICATE

The foregoing instrument was acknowledged before me this 22nd day of July, 2014, by Hua YANG. ✓ Personally known or
Produced as identification.

[Signature]
Notary Public

{Notary Seal}



28
32

THE ASSIGNEE

Agios Pharmaceuticals, Inc.

Authorized Representative

By: J. Duncan Higgons

Title: Chief Operating Officer

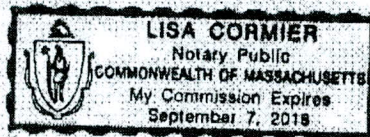
Given and signed in Cambridge, MA this 22nd day of July 2014.

NOTARIAL CERTIFICATE

On this 22nd day of July of 2014, personally appeared before me J. Duncan Higgons, whose signature is authentic and was duly identified. Duty sworn he declared that he is the COO of Agios Pharmaceuticals, Inc. I further certify that the person appearing before me is authorized to sign this document on behalf of this corporation, which according to the Charter and By Laws is a legally existing and duly organized corporation, as its duration term has not yet expired.

[Signature]
Notary Public

{Notary Seal}



22
33

Apostille

(Convention de La Haye du 5 Octobre 1961)

1. Country: United States of America
This public document
2. has been signed by **Milton A. Tingling**
3. acting in the capacity of **County Clerk**
4. bears the seal/stamp of the **county of New York**

Certified

5. at New York City, New York
6. the 17th day of February 2016
7. by Special Deputy Secretary of State, State of New York
8. No. NYC-536353
9. Seal/Stamp
10. Signature



Whitney A. Clark

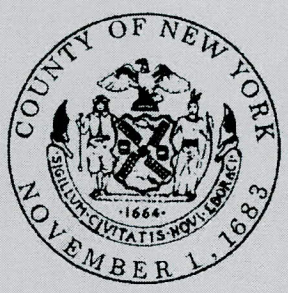
Whitney A. Clark
Special Deputy Secretary of State

State of New York }
County of New York } ss:

No. 185626

I, **Milton Adair Tingling**, Clerk of the County of New York, and Clerk of the Supreme Court in and for said county, the same being a court of record having a seal, **DO HEREBY CERTIFY THAT**

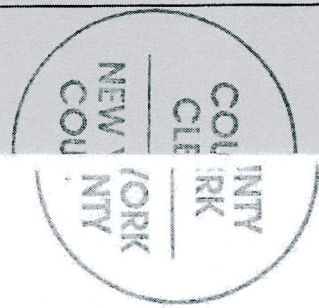
AMY LEWIS



whose name is subscribed to the annexed original instrument has been commissioned and qualified as a NOTARY PUBLIC.....
and has filed his/her original signature in this office and that he/she was at the time of taking such proof or acknowledgment or oath duly authorized by the laws of the State of New York to take the same: that he/she is well acquainted with the handwriting of such public officer or has compared the signature on the certificate of proof or acknowledgment or oath with the original signature filed in his/her office by such public officer and he/she believes that the signature on the original instrument is genuine.

IN WITNESS WHEREOF, I have hereunto set my hand and my official seal this
16th day of February, 2016

Milton Adair Tingling
County Clerk, New York County



New York
County
7, 2016

[Handwritten signature]

35

12827-843-078

VERIFICATION

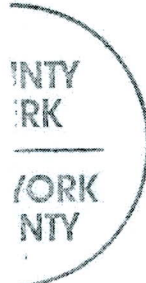
STATE OF NEW YORK)
) ss:
COUNTY OF NEW YORK)

I verify that the attached document identified as:

Assignment

is a copy of the document as executed on July 28, 2014 by Samuel V. Agresta and David Schenkein, to **AGIOS PHARMACEUTICALS, INC.** of United States and corresponding foreign rights in connection with U.S. Serial Nos. 61/861,884; 61/939,098; 61/975,448; 62/011,948 filed August 2, 2013; February 12, 2014; April 4, 2014; June 13, 2014.

Corinne Zang
Corinne Zang
JONES DAY
International Patent
Applications Supervisor



Subscribed and sworn to before me this 2nd day of
February, 2016.

Amy Lewis
Notary Public

AMY LEWIS
Notary Public, State of New York
No. 0125130070
Qualified in Kings County
Certificate Filed in New York County
Commission Expires Jan. 7, 2020

36

ASSIGNMENT

For good and valuable consideration, the sufficiency and receipt of which is hereby acknowledged, I, Samuel V. AGRESTA, (Citizenship: United States of America), 24 Coolidge Road, Lexington, Massachusetts 02420, United States of America; and David SCHENKEIN, (Citizenship: United States of America), 21 Wormwood St., Apt 622, Boston, Massachusetts 02210, United States of America, hereby:

Sell, assign and transfer to Agios Pharmaceuticals, Inc., a Delaware corporation having a place of business at 38 Sidney Street, Cambridge, Massachusetts 02139, United States of America, its successors, assigns and legal representatives, all hereinafter referred to as said Assignee, the entire right, title and interest for the United States and all foreign countries, in and to any and all inventions which are disclosed in the International Application for Patent filed with the State Intellectual Property Office of the People's Republic of China as Receiving Office of the World Intellectual Property Organization in accordance with the Patent Cooperation Treaty (PCT) on

Filed _____

under International Application Serial No. _____

and

entitled THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE,

and in and to said application and all continuing, divisional, continuation-in-part, substitute, renewal, reissue and all other applications for Letters Patent which have been or shall be filed in the United States and all foreign countries on said any and all inventions (including without limitation U.S. Provisional Application No. 61/861,884, filed August 2, 2013, and entitled THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE; U.S. Provisional Application No. 61/939,098, filed February 12, 2014, and entitled THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE; U.S. Provisional Application No. 61/975,448, filed April 4, 2014, and entitled THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE; and U.S. Provisional Application No. 62/011,948, filed June 13, 2014, and entitled THERAPEUTICALLY ACTIVE COMPOUNDS AND THEIR METHODS OF USE); and in and to all original and reissued patents which have been or shall be issued in the United States and all foreign countries on said any and all inventions including the right to apply for patent rights in each foreign country and all rights to priority; and further including any right to sue for past infringement;

Appoint, authorize and request the attorneys appointed in said application to hereafter complete this assignment by inserting in the spaces provided above the filing date and serial number of said application when known;

Agree that said Assignee may apply for and receive Letters Patent for said any and all inventions in its own name; and when requested, without charge to but at the expense of said Assignee, I agree to carry out in good faith the intent and purpose of this assignment, by executing all divisional, continuing, substitute, renewal, reissue, and all other patent applications on said any

and all inventions, by executing all rightful oaths, assignments, powers of attorney and other papers, by communicating to said Assignee all facts known to me relating to said any and all inventions and the history thereof, and generally by doing everything reasonably possible which said Assignee shall consider desirable for aiding in securing and maintaining proper patent protection for said any and all inventions and for vesting title to said any and all inventions and all applications for patents and all patents on said inventions, in said Assignee;

Request the Honorable Commissioner of Patents and Trademarks to issue said Letters Patent to said Assignee; and

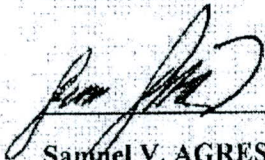
Covenant with said Assignee that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by me and that full right to convey the same as herein expressed is possessed by me.

Said Assignee does hereby acknowledge and accept the above sale, assignment and transfer of all the rights, title and interest enumerated above, including but not limited to the right to priority and the right to sue for past infringement.

In the event that the execution date, filing date and/or Application No. are not entered or are incorrect at the time Assignor(s) executed this document, Assignor(s) hereby authorize and request an attorney of Assignee to insert the information or make the correction.

38

THE ASSIGNOR



Samuel V. AGRESTA

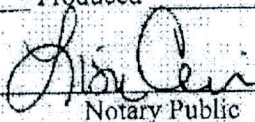
Given and signed in Cambridge, MA this 25th day of July 2014.

State of Massachusetts

County of Middlesex

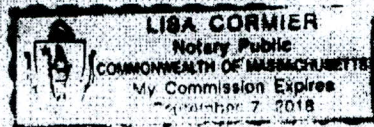
NOTARIAL CERTIFICATE

The foregoing instrument was acknowledged before me this 25th day of July, 2014, by Samuel V. AGRESTA. ✓ Personally known or Produced as identification.



Notary Public

{Notary Seal}



39
39

THE ASSIGNOR

[Signature]

David SCHENKEIN

Given and signed in Cambridge, MA this 28th day of July 2014.

State of Massachusetts

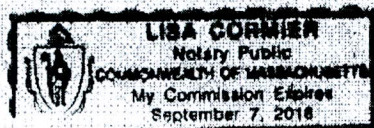
County of Middlesex

NOTARIAL CERTIFICATE

The foregoing instrument was acknowledged before me this 28th day of July, 2014, by David SCHENKEIN. / Personally known or Produced as identification.

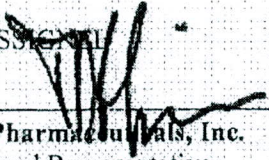
[Signature]
Notary Public

{Notary Seal}



40

THE ASSIGNMENT


Agiros Pharmaceuticals, Inc.

Authorized Representative

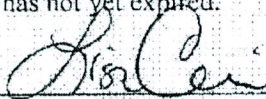
By: **J. Duncan Higgons**

Title: **Chief Operating Officer**

Given and signed in Cambridge, MA this 22th day of July 2014.

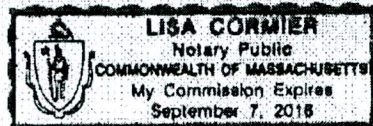
NOTARIAL CERTIFICATE

On this 22th day of July of 2014, personally appeared before me J. Duncan Higgons, whose signature is authentic and was duly identified. Duly sworn he declared that he is the CEO of Agiros Pharmaceuticals, Inc. I further certify that the person appearing before me is authorized to sign this document on behalf of this corporation, which according to the Charter and By Laws is a legally existing and duly organized corporation, as its duration term has not yet expired.



Notary Public

{Notary Seal}



41

Characterization of the Distribution, Polymorphism, and Stability of Nimodipine in Its Solid Dispersions in Polyethylene Glycol by Micro-Raman Spectroscopy and Powder X-Ray Diffraction

Received: January 12, 2007; Final Revision Received: October 23, 2007; Accepted: October 23, 2007; Published: December 7, 2007

Aristides Docoslis,¹ Krista L. Huszarik,¹ George Z. Papageorgiou,² Dimitrios Bikiaris,² Anagnostis Stergiou,³ and Emmanouel Georganakis⁴

¹Department of Chemical Engineering, Queen's University at Kingston, Ontario, Canada K7L 3N6

²Laboratory of Organic Chemical Technology, Chemistry Department, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece

³Applied Physics Laboratory, Department of Physics, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece

⁴Section of Pharmaceutics and Drug Control, Department of Pharmacy, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece

ABSTRACT

In the present study, a series of solid dispersions of the drug nimodipine using polyethylene glycol as carrier were prepared following the hot-melt method. Micro-Raman spectroscopy in conjunction with X-ray powder diffractometry was used for the characterization of the solid structure, including spatial distribution, physical state, and presence of polymorphs, as well as storage stability of nimodipine in its solid formulations. The effect of storage time on drug stability was investigated by examination of the samples 6 months and 18 months after preparation. Confocal micro-Raman mapping performed on the samples showed that the drug was not uniformly distributed on a microscopic level. The presence of crystals of nimodipine with sizes varying between one and several micrometers was detected, and the crystal size seemed to increase with overall drug content. In samples examined 6 months after preparation it was found that the crystals existed mainly as the racemic compound, whereas after 18 months of storage mainly crystal conglomerates were observed.

KEYWORDS: Solid dispersion, nimodipine, Raman spectroscopy, polymorphism

INTRODUCTION

Polymorphism describes different crystal packing arrangements of the same molecular species in its solid state, while the pseudopolymorphs are the solvate and amorphous solid-state forms. Polymorphism investigations are particularly important in drug and product development in the pharmaceutical industry, since certain properties of a formulated

product, such as bioavailability and stability, are often directly related to the physicochemical properties of the existing polymorphs in the formulation.¹⁻³

X-ray powder diffractometry (XRPD) and solid-state nuclear magnetic resonance are used in the identification of polymorphs, while spectroscopic techniques such as infrared (IR) and Raman can supply information on the short-range structure of polymorphs.^{4,5} These techniques can also be used for the quantification of polymorphic composition and degree of crystallinity.⁶ The fundamental molecular vibrations are different for each polymorph because of the different packing arrangements, providing a unique "fingerprint" in the form of a spectrum.^{7,8} Raman spectroscopy has 3 significant advantages over traditional spectroscopic techniques, such as Fourier transform infrared (FT-IR) spectroscopy. First, it requires no sample preparation. Second, coupling to an optical microscope allows the study of small particles within nonhomogeneous solid sample matrices. Finally, Raman spectra can be obtained noninvasively, even within a sealed transparent container.⁹ If the incident light is transmitted through an optical microscope (magnification $\times 100$) before reaching the sample, the light scattered from an area of only a few micrometers can be collected.^{7,8,10} The spatial resolution can be further improved in confocal systems, where the collected light passes through a pinhole before reaching the detection system, allowing collection of spectra from a sample volume smaller than $1 \mu\text{m}^3$.

Nimodipine is a drug of the 1,4-dihydropyridene type that can be used for the prevention and treatment of ischemic neurological deficits.¹¹⁻¹⁴ The substance is a racemic mixture of 2 optical antipodes and shows 2 polymorphic forms.¹¹ Modification I (mod I) is the racemic compound, while modification II (mod II) is the conglomerate. Nimodipine is practically insoluble in water and exhibits low bioavailability after oral administration.¹¹ Therefore, improving the dissolution characteristics of nimodipine from its oral solid dosage forms becomes a major goal. To date the literature on solid dispersions of nimodipine has been limited.¹⁵⁻¹⁷

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42
42

The development of effective new formulations requires detailed investigation of nimodipine's polymorphism,^{11,12,14} particularly since nimodipine dispersed in a solid carrier may exist simultaneously in 2 crystal modifications and as an amorphous material.¹⁷

Solid dispersions can be used to increase the dissolution rate of a drug with low aqueous solubility, thereby improving its bioavailability.¹⁸⁻²¹ One of the most critical aspects in the preparation of such formulations is the ability to assess (and, subsequently, control) the physical state of the drug and its particle size distribution, 2 variables that can significantly influence the drug dissolution rate. Typically, X-ray diffraction (XRD) and scanning electron microscopy (SEM) are used for this task. However, in the case of amorphous drug dispersions, SEM is unable to unequivocally evaluate the drug distribution in the matrix. Confocal micro-Raman spectroscopy, which can provide solid-state characterization of both amorphous and crystalline materials, can be useful in achieving this goal. To the best of our knowledge, the technique has not been used until now in the complete investigation of solid dispersions of drugs, except in 1 study.²²

The objective of this work is to explore the potential of confocal micro-Raman spectroscopy to qualitatively and quantitatively characterize the solid state of a drug, as well as its dispersion—physical form and spatial distribution—in a polymer matrix (inert drug carrier). The scope of the study is enhanced by including an investigation of the drug's stability (physical state and polymorphism) in the mixtures during long-term storage. The samples examined were solid dispersions of nimodipine in polyethylene glycol (PEG) 4000, prepared according to the hot-melt method. In addition to micro-Raman spectroscopy, XRPD, differential scanning calorimetry (DSC), and hot-stage microscopy (HSM) are used to provide physicochemical characterization of the samples.

EXPERIMENTAL

Materials and Equipment

Micronized nimodipine with an assay content of 101.2%, a melting point of 125°C to 128°C, an aqueous solubility of ~0.5 mg/L, and free solubility in ethanol was supplied by UQUIFA (Barcelona, Spain). The particle size distribution was determined using a Malvern Mastersizer S (633 nm) (Worcestershire, UK) and found to range from ~1 μ m (or less) to 29 μ m (10% up to 1 μ m, 50% between 1 and 9 μ m, and 40% between 9 and 29 μ m). PEG 4000 with a molecular weight of 3898 g/mol (calculated by OH end groups), a T_m of 54°C (DSC analysis), a moisture content <0.5% (thermogravimetric analysis), and a viscosity of 118 mPa·s (at 20°C, 50% relative humidity [RH]) was obtained from Clariant (Sulzbach, Germany). All other materials and reagents were of analytical grade.

Methods

Preparation of Solid Dispersions

The solid dispersions were prepared by the hot-melt technique. Preliminary HSM tests showed that the temperature for complete dissolution of the nimodipine crystals in the melt of PEG increases with drug content and heating rate, and it was verified that at temperatures between 65°C and 130°C nimodipine starts to dissolve into PEG melt. Thus, physical mixtures of nimodipine and PEG were heated during stirring in a reaction tube immersed in an oil bath to 130°C, under an argon atmosphere. The mixtures were then held to this temperature to ensure that the drug was completely melted and a homogeneous and transparent solution was obtained. Solid dispersions with nimodipine/PEG 10/90 wt/wt, 20/80 wt/wt, 30/70 wt/wt, 40/60 wt/wt, 50/50 wt/wt, and 100/0 wt/wt (control) were prepared. Next, the tubes were immersed in a water bath to quench the melt. The prepared samples were stored at 25°C in desiccator.

To evaluate the effect of long-term storage on the physical structure of nimodipine, the solid dispersions were stored up to 18 months at 25°C and 60% RH.

DSC

DSC studies of the prepared samples were conducted immediately after preparation as well as after storage for 6 months. A Perkin Elmer Pyris 1 DSC (Shelton, CT) equipped with an Intracooler 2P cooling accessory was used. Samples of 5 mg were placed in standard aluminum pans and sealed with a lid. Heating scans of 20°C/min or 100°C/min were applied with a nitrogen purge of 20 mL/min. Fast heating rates are preferred, to prevent changes during scanning.²³

XRPD

XRPD study of the samples after storage for 10 days as well as after 6 and 18 months was performed over the range 2 θ from 5° to 60°, at steps of 0.05°. A Philips PW1710 powder diffractometer (Eindhoven, The Netherlands) with CuK α nickel-filtered radiation was used.

Confocal Micro-Raman Spectroscopy

Raman studies were performed using a HORIBA Jobin Yvon (Edison, NJ) micro-Raman spectrometer (model: LabRAM) equipped with a 632 nm He/Ne laser source, 1800 1/nm grating, and an Olympus BX41 microscope system. Collection of spectra in back-scattering mode was performed at room temperature under the following conditions: $\times 100$ microscope objective, 50 μ m pinhole size, 300 μ m slit width, and 5 second exposure time. Each spectrum represents the average of 2 measurements. XY scanning (2D

mapping) of the samples was performed at the conditions stated above with the aid of a computer-controlled motorized stage at a step of 0.3 μm in both X- and Y-directions. The effect of surface topography was avoided by automatically focusing the laser beam at each point using the "auto-focus" function of the software (LabSpec 4.1 HORIBA Jobin Yvon [Edison, NJ]). The samples were first ground to a fine powder and then spread flat on a glass microscope slide.

HSM

A polarizing optical microscope (Nikon, Optiphot-2, Tokyo, Japan) equipped with a Linkam THMS 600 heating stage, a Linkam TP 91 control unit, and a Jenoptic ProgRes C10plus camera with Capture Pro 2.1 software was used for HSM observations.

RESULTS AND DISCUSSION

Characterization of the Modifications of Nimodipine

Nimodipine is a substance that can be obtained in either the amorphous or the crystalline state depending on the treatment and storage conditions. Two different crystal modifications of the crystalline substance are found.¹¹ Commercial nimodipine samples are in fact racemic mixtures of the 2 optical antipodes.^{11,13} An interesting subject of chemical research has been the relationship between the racemic and chiral crystals of 2 opposite and resolvable enantiomers (*d* and *l*). Three crystal forms may appear for substances showing optical antipodes: the racemate or racemic compound, a crystal containing both *d* and *l* in the same unit cell; the enantiomorph, a chiral crystal of *d* or *l*; and the conglomerate, an equimolar mixture of the *d* and *l* enantiomorphs. Crystal mod I of nimodipine refers to the racemic compound, while mod II refers to the conglomerate.¹¹ Mod II is favored by crystallization at room temperature, as well as by crystallization in the presence of isopropanol or ethanol traces. If the homogeneous mixture of the optical antipodes cocrystallizes, the racemic compound is formed. If the antipodes are locally separated, crystallization results in a mixture of the crystals of the pure antipodes (conglomerate). Below 88°C the less soluble mod II is the most stable. The system is enantiotropic and crystals of mod I transform to mod II at room temperature.¹¹ To achieve dissolution rates that are as high as possible, the drug must be kept in the amorphous state or, if it partly crystallizes in the formulations, then its transformation from mod I to mod II crystals must be prevented.

Figure 1 presents a series of wide-angle XRPD patterns for various nimodipine samples. The pattern of the original ("as received") drug sample used in this study corresponds to that of nimodipine crystal mod I. The one obtained after

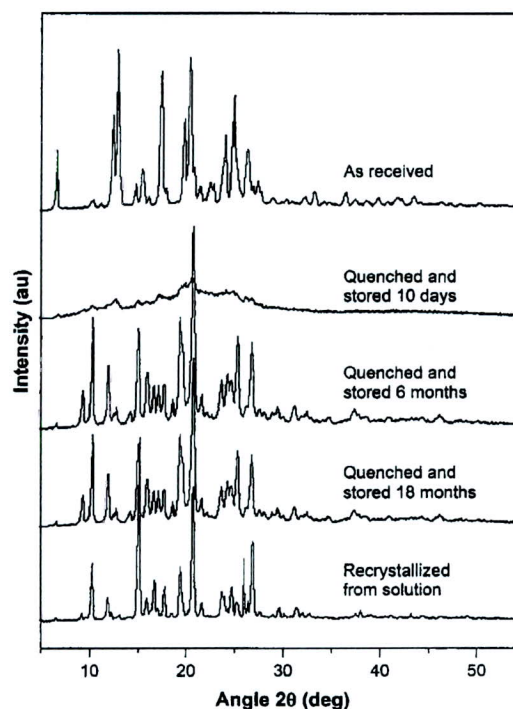


Figure 1. X-ray powder diffractometry patterns of various nimodipine samples.

crystallization from solution in ethanol corresponds to the nimodipine crystal mod II. The XRPD patterns for the 2 crystal modifications are very similar, except for the crystalline reflections at low angles 2θ : for example, characteristic peaks at $2\theta = 6.7^\circ$ for mod I, and at $2\theta = 10.40^\circ$ for mod II can be easily observed. Furthermore, the pattern of a melt-quenched nimodipine sample even after storage for 10 days at room temperature shows very low crystallinity. This finding indicated that nimodipine can be obtained in an essentially amorphous state from a melt-quenching procedure. The DSC study showed a glass transition temperature $T_g = 20^\circ\text{C}$ for the drug. It was found that the substance, in contrast to many other drugs, does not show crystallization during cooling from the melt, or during heating an amorphous sample from room temperature up to the melting point (126°C), at least when the cooling or the heating rate is faster than $5^\circ\text{C}/\text{min}$. The melt-quenched sample crystallized during long-term storage for 6 months, and its pattern appears in Figure 1, along with the pattern for after 18 months. The reflections were basically those of crystal mod II.

Confocal micro-Raman spectroscopy was used to study the polymorphism of nimodipine. XRPD relies on the supramolecular (physical) structure—that is, the extent of 3-dimensional order—to distinguish between the crystalline and the amorphous state of the drug and the specific characteristics of the crystalline structure. Despite the fact that the crystal

44

modifications are the result of the arrangement of the antipodes, XRPD cannot directly detect differences on a molecular level. Micro-Raman spectroscopy, however, allows examination of the intermolecular (chemical) structure of a substance and therefore enables the characterization of not only the crystalline drug but also the amorphous drug.

The 2 crystal modifications of nimodipine can be distinguished by means of vibrational spectroscopy, as they exhibit different (Raman and IR) spectra, meaning different intensities at the characteristic peaks of the drug.^{11,17} As can be seen in Figure 2, the peak intensity at 1642 cm⁻¹ (C=C bond stretch of the dihydropyridine ring) for the racemic crystal (mod I) is significantly lower than that at 1347 cm⁻¹ (symmetric stretching vibrations of the -NO₂ group²⁴). As a result, this intensity ratio for mod I is much larger than 1. In contrast, the same ratio for the homochiral crystal (mod II) gives a value much smaller than unity. Interestingly, the intensities of the 2 peaks are almost balanced in the spectrum of the amorphous drug, resulting in a $I_{1347} \div I_{1642}$ ratio close to unity. The values of the intensity ratios correspond-

ing to each physical state of the pure drug are shown in Table 1. The tabulated mean values are for 100 measurements taken from each sample. This significant variation in the $I_{1347} \div I_{1642}$ ratio between the amorphous and 2 crystalline states of the drug may be useful in determining the physical state of nimodipine in its solid dispersions.

Characterization of Solid Dispersions

In his review article, Craig underlined 4 key problem areas in the formulation and use of solid dispersions: the solid structure, the mechanism by which dissolution enhancement occurs, the stability of the dispersions during storage, and the poor understanding of the in vitro/in vivo correlation.²⁰ The first 3 areas are related to the physical characteristics of the dispersions and, consequently, to the manufacturing process employed for their preparation. As mentioned in the introduction, the objective of this study was to explore the capabilities of micro-Raman spectroscopy in the characterization of the solid state of nimodipine in a series of solid

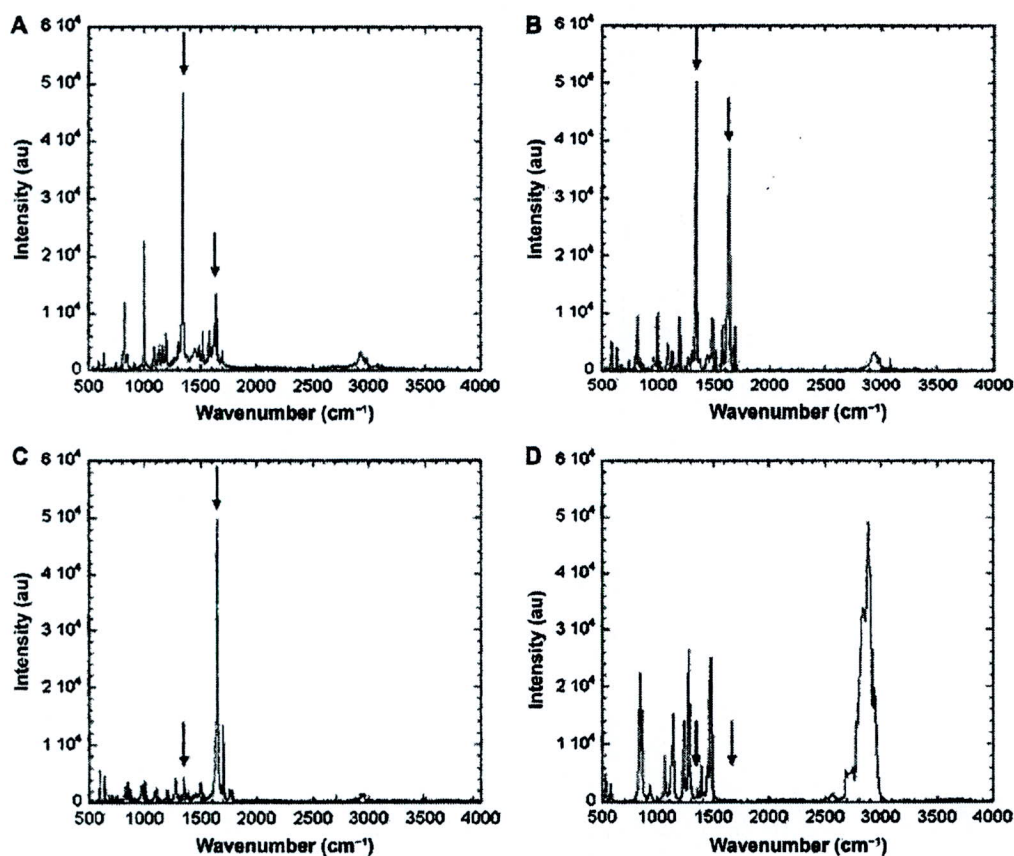


Figure 2. Raman spectra of nimodipine existing in different forms: (A) crystal of modification I; (B) amorphous phase; (C) crystal of modification II; (D) Raman spectrum of pure polyethylene glycol. For (A), (B), and (C), the polymorphs can be distinguished on the basis of the peak intensities at 1347 and 1642 cm⁻¹, as indicated by the arrows. In (D), the arrows indicate that no peaks exist at the above wavenumbers.

45

Table 1. Intensity Ratios Corresponding to the 3 Types of Nimodipine Examined*

Phase	Structure	Peak 1 (cm ⁻¹)	Peak 2 (cm ⁻¹)	Intensity Ratio, R (=I ₁ ÷ I ₂)	
				Mean	SD
Modification I	Crystalline	1347-1348	1642-1643	3.67	0.58
Amorphous	Amorphous	1349-1350	1646-1648	1.22	0.17
Modification II	Crystalline	1348-1349	1643-1644	0.171	0.035

*The columns labeled Peak 1 and Peak 2 indicate the wavenumber range of 2 characteristic band assignments.

dispersions in PEG prepared by the hot-melt technique. Since nimodipine has no detectable elements such as Cl, F, Br, or Na, it is not possible for its particles to be distinguished with SEM–energy dispersive spectroscopy or transmission electron microscopy, which our team has used in previous studies.²⁵⁻²⁷ Additionally, SEM can detect only well-formed crystal particles and is unable to give information on particle distribution in cases where the dispersed drug is in the amorphous state. On the other hand, powder XRD is used to study collectively the bulk of a sample, since it detects crystalline drug over a flat sample of significant surface area ($\sim 3 \times 3 \text{ cm}^2$), giving an averaged picture for crystallinity and crystal modifications; however, it cannot detect, or provide information on, the amorphous phase. For this reason micro-Raman spectroscopy was selected as an alternative technique that can possibly provide a more complete sample characterization by combining the capabilities of the previous 2 methods. By surface scanning an area, confocal micro-Raman spectroscopy can offer selective image contrast of each molecular component of a mixture on a plane, while optical microscopy provides only a surface mapping of the whole mixture, where the contrast appears usually in terms of reflective index or reflectivity. The principle of Raman imaging consists of scanning the surface at a fixed wavenumber characteristic of a component of the mixture (eg, stretching vibrations of the NO₂– group of nimodipine). Each time the incident monochromatic light meets the sample, the intensity of a peak at a Raman shift characteristic of the component of interest is collected and measured, while those of the other components are omitted.⁸

Since the FT-Raman and micro-Raman techniques have begun to be used in the study of pharmaceuticals only recently, a limited number of studies currently exist in the literature.²⁸⁻³⁴ Before now, mapping has been used in only 1 study of solid dispersion systems.²²

The preparation and storage conditions of the solid dispersions play a critical role, as changes may alter the dissolution characteristics of the active ingredients over time.³⁵⁻⁴¹ Drugs dispersed in the amorphous phase are expected to be more soluble than the crystalline or polymorphic forms. However, amorphous drugs are less stable and more sensi-

tive to physical alterations during storage. Thus, examination of the morphology of the drug and the polymer matrix in the solid dispersions is very important to test the possible effects of storage. Methods for monitoring the stability of the amorphous state of the drug dispersed in the polymer have been explored for a long time. DSC, differential thermoanalysis, solution calorimetry, IR spectroscopy, XRPD, and only recently FT-Raman spectroscopy have been used.^{42,43} XRPD and micro-Raman spectroscopy are used comparatively in the present study.

Identification of the crystal modifications of nimodipine in its mixtures with PEG was attempted by using as criterion the intensity ratio of the Raman bands at 1347 cm⁻¹ and 1642 cm⁻¹ (I₁₃₄₇ ÷ I₁₆₄₂). In light of the fact that the value of this ratio was so distinctly different among the physical states of the drug in its pure form, we examined whether it could also serve as a tool for the quick characterization of nimodipine in its solid dispersions. Figures 3A and 3B show examples of (XY) micro-Raman scans performed on samples with nimodipine/PEG weight ratios 10/90 and 50/50 wt/wt, respectively, 6 months after their preparation. Figures 3C and 3D show scanning results from samples of the same compositions (90/10 and 50/50, respectively) after 18 months of storage. The color bar in the images indicates the value of the intensity ratio I₁₃₄₇ ÷ I₁₆₄₂. In the scans obtained from samples after 6 months of storage, areas of various size that displayed a very high intensity ratio (yellow/white, I₁₃₄₇ ÷ I₁₆₄₂ ≥ 3.5) were seen to be surrounded by continuous areas with values in the range 0.25 < I₁₃₄₇ ÷ I₁₆₄₂ < 3.5. As can be seen in Figure 3A, dark blue areas (I₁₃₄₇ ÷ I₁₆₄₂ ≤ 0.25) were found to coexist with areas displaying higher intensity ratios. The scans obtained from samples stored for 18 months had a totally different appearance (Figures 3C and 3D). Here, the yellow areas had almost disappeared, while dark blue areas were more frequent and larger than before. The above observations were consistent among all the micro-Raman scans (5 for each concentration).

At first glance, a challenge in interpreting these scans arises from the big disparity of the intensity ratio values over the whole sample, especially in the large areas represented by intermediate colors (0.25 < I₁₃₄₇ ÷ I₁₆₄₂ < 3.5) that cannot be

46

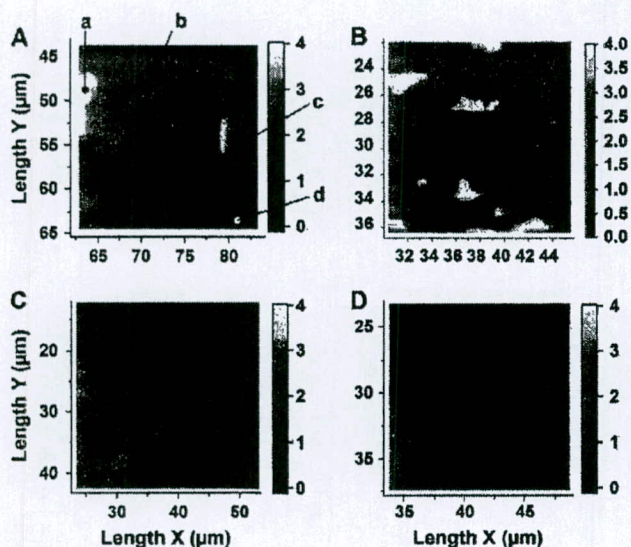


Figure 3. Micro-Raman XY scans of samples prepared by the melt method. Scans (A) and (B) correspond to nimodipine/PEG 10/90 and 50/50 wt/wt, respectively, and were obtained 6 months after sample preparation. Scans (C) and (D) correspond to nimodipine/PEG 10/90 and 50/50 wt/wt, respectively, and were obtained 18 months after sample preparation. The color map indicates spatial variation of the intensity ratio $I_{1347} \div I_{1642}$. PEG indicates polyethylene glycol. Points a, b, c, and d in Figure 3A correspond to spectra a, b, c, and d in Figure 4E-H.

readily classified as corresponding to completely crystalline or amorphous nimodipine. To investigate the physical state of nimodipine in its solid dispersions with PEG and whether a meaningful relationship exists between this and the value of the $I_{1347} \div I_{1642}$ intensity ratio, a close examination of the Raman spectra corresponding to regions of different color in the scans was performed. Identification of the physical state of nimodipine in the mixture was attempted by comparing the spectra obtained from mixtures with those of pure nimodipine, as seen in Figure 4. The first row (Figures 4A, 4B, 4C, and 4D) depicts the Raman signatures of pure nimodipine (and PEG) in selected spectral windows where differences between the racemic and homochiral crystals of the drug can be observed. Figure 4A shows that mod I exhibits a single band at 1198 cm^{-1} (corresponding to the stretching vibration of the C-C bond between the aromatic and pyridine rings²⁴), whereas in the spectrum of mod II a split of this band had occurred. The amorphous material showed a distinct band at 1197 cm^{-1} and a poorly resolved peak at slightly higher wavenumbers. Figure 4B shows the peaks assigned to the symmetric stretching vibrations of the $-\text{NO}_2$ group. This peak exhibited high intensity at 1347 cm^{-1} in the spectra of mod I. In the mod II spectrum, the peak was significantly weaker and slightly shifted to higher wavenumbers ($1348\text{--}1349 \text{ cm}^{-1}$). The amorphous material displayed a broad band centered at $\sim 1348 \text{ cm}^{-1}$. The relation

between the bands of mod I (1642 cm^{-1}) and mod II (1644 cm^{-1}) was the opposite in Figure 4C (C=C stretch bond), where the latter exhibited a band of significantly higher intensity. The band for the amorphous material was broad and centered at 1647 cm^{-1} , and was of higher intensity than that of mod II. Finally, in Figure 4D the bands corresponding to the stretching vibrations of the $>\text{C}=\text{O}$ groups of the molecule are shown. The bands in this part of the spectrum were typically weak, although that of mod II (1699 cm^{-1}) was generally significantly larger than the others (1700 cm^{-1}). An additional, less intense, band (1692 cm^{-1}) could be observed in the spectrum of mod I.

The spectra of pure nimodipine described above were subsequently used as reference for identifying the physical state of the drug in its solid dispersions with PEG. The examination involved over 200 spectra collected from various points in Raman scans obtained from mixtures with concentrations 10 to 50 wt% in nimodipine. An example is given in Figures 4E-4H, which show the corresponding spectra of the points marked as A through D in Figure 3A. As can be seen, the Raman signatures (band shapes and locations) of points A ($I_{1347} \div I_{1642} \geq 3.5$) and D ($I_{1347} \div I_{1642} \leq 0.25$) were very similar to those of mod I and mod II, respectively. This finding was consistent (and exclusive) for all points collected from areas with intensity ratios outside the range 0.25 to 3.5. Specifically, the examination of 20 samples from areas with a ratio greater than 3.5 (yellow-colored areas) gave an average value of 3.84 (SD ± 3.67 ; median 3.69). The examination of 20 samples from areas with a ratio less than 0.25 (dark blue areas) gave an average value of 0.18 (SD ± 0.05 ; median: 0.19). On the basis of this result it can be concluded that the ratio $I_{1347} \div I_{1642}$ can be used as a criterion for identifying the presence of crystals and their modification in the PEG/nimodipine solid dispersions. The Raman signatures of points from regions with a ratio within the range $0.25 < I_{1347} \div I_{1642} < 3.5$ were also examined. The Raman spectra collected from these regions did not fully conform to any of the spectra observed for the pure crystalline or amorphous nimodipine. Points B (green area) and C (light blue) were 2 examples of that case. From Figures 4E to 4H it can be seen that the spectrum of point B had a lot of similarities with that of mod I, but the shape and location of its band at 1700 cm^{-1} resembled that of the amorphous material. Raman spectra collected from samples of 1 wt% nimodipine in PEG (results not shown) gave the same peak assignments and $I_{1347} \div I_{1642}$ ratios as those obtained from amorphous pure nimodipine. It can therefore be inferred that both forms of nimodipine (amorphous and mod I) might be present in this location. On the other hand, point C followed loosely the characteristic bands of mod II, suggesting that some of the drug in this area was present in the form of crystals of mod II. From Figure 4E it can be seen that the spectra of these 2 points also contained bands that belonged to the molecules

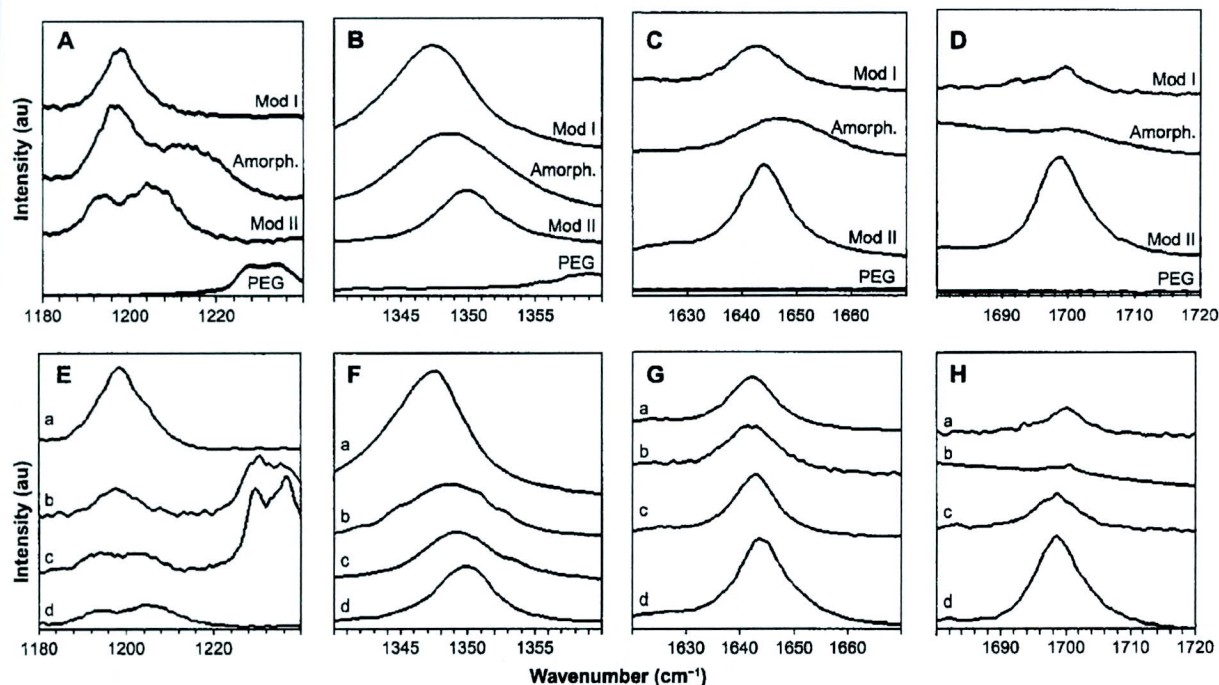


Figure 4. (A), (B), (C), and (D) Details of Raman spectra obtained from samples of pure nimodipine; (E), (F), (G), and (H) nimodipine/PEG solid dispersions. Mod indicates modification; Amorph, amorphous; PEG, polyethylene glycol. Spectra a, b, c, and d in Figure 4E-H correspond to points a, b, c, and d in Figure 3A.

of PEG, an indication that the drug did not exist in pure form. It should also be mentioned that, for both points, the characteristic peaks of their spectra were found to be less intense than those corresponding to the crystalline modifications and also slightly shifted to different frequencies. Intensity attenuations and band shifts result from local variations in drug structure and composition and indicated that the intermolecular interactions of the nimodipine with its surroundings were not the same everywhere. In fact, shifts in the bands of nimodipine (occasionally up to 4 cm⁻¹) were frequently observed to occur in spectra collected from different locations in the scans. Notably, in some of the spectra (approximately 30 out of 200) shifts in the band assigned to NO₂-stretch (normally appearing at 1642-1644 cm⁻¹) were also found, resulting in erroneous calculations of the $I_{1347} \div I_{1642}$ ratio. Taking into account all these discrepancies, it was concluded that the values of the ratio $I_{1347} \div I_{1642}$ in the range of 0.25 to 3.5 indicated material existing in a heterogeneous state, but this parameter could not be used alone for an in-depth interpretation of the physical state of the drug in the mixtures.

In summary, we showed that examination of spectra corresponding to $0.25 < I_{1347} \div I_{1642} < 3.5$ revealed that nimodipine in these regions coexisted with PEG and may be present simultaneously in more than 1 form. The composition of these regions, which was found to vary with location, can be best described as heterogeneous. Identification of

domains within these areas where nimodipine existed in only a single form could not be performed, as the size of these domains was below the resolution capabilities of the instrument used in this study. Therefore, we can only speculate that these areas contained crystals of nimodipine of molecular to submicron-sized dimensions and/or amorphous regions formed in the presence of PEG. Detailed analysis of the Raman spectra, for instance, through peak deconvolution and subsequent integration of the area under each Raman peak, can possibly provide more information on the composition and amounts of nimodipine present in each domain, but this analysis often proves laborious.

The conclusions drawn from this analysis can now be used toward an interpretation of the micro-Raman maps of Figure 3. Having established that the ratio $I_{1347} \div I_{1642}$ may be used for the identification of crystalline domains of the drug in the PEG/nimodipine melts, white/yellow domains ($I_{1347} \div I_{1642} \geq 3.5$) in the scans can be taken to represent crystals of mod I, while dark blue domains ($I_{1347} \div I_{1642} \leq 0.25$) can be taken to represent crystals of mod II. In the scans corresponding to samples examined 6 months after preparation, crystals with sizes from one to several micrometers could be seen. Overall, it appeared that the crystals (mod I or mod II) of nimodipine seemed to coexist in highly distributed and discontinuous microdomains throughout the sample volume. Several scans (5 per concentration) performed on

48

these samples indicated a trend of increasing crystal size with overall drug content. This semiquantitative observation was also supported by HSM studies (not shown) performed above 60°C so that the melting of the polymer matrix (PEG 4000) would allow observation of the size of the drug crystals. These observations could be rationalized in terms of an increasing degree of crystallinity as a result of supersaturation observed with increasing drug content. An estimate, obtained through XRPD data analysis, showed that the drug's crystallinity was 34, 43, 55, 64, and 67 wt% in solid dispersion containing 10, 20, 30, 40, and 50 wt% nimodipine, respectively. The crystallinity was assessed by using as standards physical mixtures of nimodipine/PEG containing the drug in its "as received" form (pure crystalline nimodipine). The results were obtained by first subtracting from the XRPD patterns the peaks corresponding to crystal reflections of PEG and then integrating the area under the peaks of crystalline nimodipine.

Although crystals could be seen in the micro-Raman scans, the majority of the mapped areas consisted of heterogeneous domains ($0.25 < I_{1347} \div I_{1642} < 3.5$) representing mixtures of nimodipine with PEG. As mentioned earlier, Raman spectra collected from these domains provide indications that the drug existed in different physical states, the length scales of which (molecular to submicron) are too small to be resolved with the present technique.

Samples with drug concentration 10 and 50 wt% were also examined after 18 months of storage (Figures 3C and 3D, respectively). The appearance of these samples was in stark contrast to that of the samples after storage for 6 months. Specifically, areas where the drug existed in crystalline mod II seemed to have increased, while crystals of mod I had almost disappeared. As discussed previously, the scans showed that the majority of the sample is occupied by heterogeneous domains.

Examination of the drug stability with time was also performed by means of XRPD. The patterns of the solid dispersions were recorded at 3 different times: 10 days, 6 months, and 18 months after preparation of the samples. The study showed that no substantial change occurred in the sample over the period between the first 2 measurements. However, the XRPD traces recorded at 18 months were quite different compared with the initial measurements, possibly because of crystal modification of the drug. The patterns after 10 days of storage showed peaks of nimodipine crystal mod I, while the peaks in the patterns after 18 months were in principle of mod II, though there were some residual peaks corresponding to mod I. For pure nimodipine, crystallization below 88°C favors appearance of mod II. Our tests in the case of pure melt-quenched nimodipine showed that in the XRPD pattern after 18 months of storage at 25°C only peaks for mod II crystals appeared. Although the drug appeared to

be effectively stabilized in the dispersion in the polymer for times up to 6 months, the storage period of 18 months was long enough for changes in the crystalline structure of the drug to occur. In general, the XRPD results were in qualitative agreement with those obtained from Raman mapping in terms of the changes in the crystalline modification of the drug with time.

CONCLUSIONS

In the present study, characterization of the distribution and physical state of the drug nimodipine in its solid dispersions with PEG was attempted by means of confocal micro-Raman spectroscopy and powder XRD. Two-dimensional Raman maps displaying the spatial variation of the intensity ratio of 2 characteristic peaks of the Raman spectrum of nimodipine ($I_{1347} \div I_{1642}$) were used for the examination of samples with overall nimodipine content between 10 and 50 wt%. The mapping results revealed that part of the drug existed in the form of crystals with sizes from one to several micrometers. The majority of the mapped areas, however, were found to constitute heterogeneous regions where nimodipine coexisted with PEG. Raman spectra obtained from points within these regions were not consistent and seemed to vary randomly with location, which suggested that nimodipine was not uniformly distributed and might, locally, be present simultaneously in more than 1 form. Further information on the distribution and physical state of nimodipine inside these heterogeneous regions may be possibly obtained through detailed analysis of the collected spectra; this laborious task will be the objective of future research.

Micro-Raman spectroscopy and XRPD were also used to study the effect of storage time on the stability of the drug, and the observed results were in qualitative agreement. Specifically, in samples examined 6 months after preparation it was found that the drug crystals were mainly heterochiral (mod I). Examination of the same samples after 18 months of storage showed the presence of only homochiral crystals (mod II), indicating that this storage period was long enough for the transformation in the crystalline structure of the drug to occur.

ACKNOWLEDGMENTS

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49
49

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Crystal Polymorphism in a Carbamazepine Derivative: Oxcarbazepine

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Abstract

Although crystal polymorphism of carbamazepine (CBZ), an anticonvulsant used to treat epilepsy, has been known for decades, the phenomenon has only recently been noted for its keto-derivative oxcarbazepine (OCB). Here it is demonstrated that OCB possesses at least three anhydrous polymorphs. Although all forms are morphologically similar, making differentiation between crystal modifications by optical microscopy difficult, powder X-ray diffraction, Raman spectroscopy, and thermomicroscopy show distinctive differences. These techniques provide an efficient method of distinguishing between the three polymorphs. The crystal structure of form II of OCB is reported for the first time and the structure of form I has been redetermined at low temperature. Remarkably, both the molecular conformation and crystal packing of form II are in excellent agreement with the blind prediction made in 2007.

Keywords

polymorphism; X-ray diffractometry; Raman spectroscopy; crystal structure; calorimetry (DSC)

INTRODUCTION

Polymorphism occurs when a molecule is able to form multiple crystal structures where the packing and/or molecular conformation differs between structures.¹ Although this is far from a universal occurrence in organic compounds,² crystal polymorphism has considerable technical importance in industry, especially among pharmaceuticals, where the presence of different structures can affect dissolution rate, solubility, and bioavailability, among other physical characteristics.¹ Despite being highly investigated, the phenomenon of polymorphism continues to present new challenges. In molecular solids, hydrogen bonding and molecular flexibility are thought to be factors contributing to increased propensity towards polymorphism,³ yet predicting the presence of polymorphic behavior, much less the structure of the polymorphs, remains an outstanding challenge. The conditions under which a crystal is grown clearly affect the crystal structure in polymorphic systems. Recently, insoluble polymers have been utilized to selectively control the crystallization of one polymorph over another.⁴ The use of these polymeric heteronuclei has become an efficient and effective tool for screening for polymorphic behavior and the discovery of new polymorphs^{4,5} offering the potential to shed light on this fundamental issue in solid-state chemistry. Here we disclose our success in applying polymer-induced heteronucleation to solid form discovery for oxcarbazepine.

Carbamazepine (CBZ) (Fig. 1), an important anticonvulsant, exhibits four structurally characterized polymorphs,⁶ whereas its dihydro derivative (10,11-dihydrocarbamazepine) is trimorphic.^{7–9} Its keto-derivative, oxcarbazepine (OCB) (Fig. 1), also used as an anticonvulsant, is believed to also be polymorphic and patent literature claims a total of five polymorphs and a solvate albeit with levels of characterization inadequate to prove that these are novel forms.^{10,11} The characterization of three anhydrous polymorphs is reported herein. The commercially available form I crystallizes in the monoclinic space group $P2_1/c$, with cell constants of $a = 5.276(2)$ Å, $b = 9.307(4)$ Å, $c = 24.841(7)$ Å, and $\beta = 95.64(2)^\circ$.¹² The possibility of other structures for OCB has been suggested by computational studies,¹³ although these have yet to realize experimental verification. One form proposed by Cabeza et al. appears to have a similar powder X-ray diffraction pattern to that of a form suggested in the patent literature.^{10,11} The present study reveals two additional forms of OCB, including one that matches the prediction of Cabeza et al., making it, at least, a trimorphic system. This observation contributes to the mounting evidence that the basic backbone of carbamazepine and urea functionality constitute a polymorphophore.¹⁴

The two newly discovered forms of OCB reported herein, form II and form III, along with form I have been characterized by Raman spectroscopy, differential scanning calorimetry (DSC), thermomicroscopy, and powder X-ray diffraction (PXRD). The structure of form I was redetermined at low temperature. The crystal structure of form II was solved and found to be in good agreement with that predicted by Cabeza et al.¹³

EXPERIMENTAL

Materials

Commercial OCB was obtained from Orgamol (Eviornaz, Switzerland). Toluene (ACS grade) and methanol (ACS grade) were purchased from Fisher Scientific (Pittsburgh, PA). Ethylene/vinyl acetate copolymers and high-density polyethylene were purchased from Scientific Polymer Products, Inc. (Ontario, NY).

Preparation of OCB Polymorphs

Form I of OCB was obtained directly from the bottle and from the evaporation of a methanol solution. Various concentrations were used, with higher concentrations yielding larger, better quality crystals. Form II was produced by dissolving 25.0 mg of OCB in 20 mL of toluene and heating the solution to 115°C until homogeneous. The solution was then allowed to cool to room temperature or placed in an ice bath until crystals were formed. The small needles (in most cases more powder-like than individual crystals) were collected after about an hour. Seeding of supersaturated toluene solutions (2.5 mg/mL) of oxcarbazepine at room temperature with form II seeds consistently yielded bulk form II, whereas the cooling of the solution alone yielded either form I or form II. Form III was obtained through the evaporation of methanol solutions (5 mg/mL) in the presence of ethylene/vinyl acetate copolymers or high-density polyethylene at room temperature.

Powder X-Ray Diffraction

PXRD patterns were obtained on a Bruker D8 Advance diffractometer operating at 40 kV and 40 mA with a copper source between 3 and 50° 2 θ with a step size of 0.020°. The step time for forms I and II was 5 s and that for form III was 30 s. Samples were measured directly on a glass slide at room temperature.

Single Crystal X-Ray Diffraction

All measurements were made on a Rigaku R-Axis SPIDER diffractometer with an image plate detector using graphite monochromated Cu-K α radiation (1.5406 Å). The data

collection was made at 95 K with the sample mounted on a MiTeGen MicroMount™. The structure was solved by direct methods.¹⁵ All calculations were performed using the CrystalStructure crystallographic software package¹⁶ except for refinement, which was performed using SHELXL-97.¹⁷

Raman Spectroscopy

Raman spectra were recorded on a Renishaw inVia Raman Microscope equipped with a 20× objective and utilizing a 633 nm HeNe laser. The scan range was 3600–100 cm⁻¹ using three scans of length 60 s per spectrum. A silicon standard was used to calibrate the instrument.

Infrared Spectroscopy

IR spectra were obtained with a Perkin-Elmer AutoIMAGE FTIR microscopy system in transmission mode using 100 scans per spectrum.

Thermomicroscopy

A Mettler Toledo PP82HT hot stage using an FP90 control processor was used for thermomicroscopy. The sample was observed under polarized light with a Leica DMLP microscope. Samples were heated from 30 to 220°C at a rate of 5°C/min. Forms II and III were also heated until each fully transformed and then allowed to cool. The transformation product was then identified using Raman spectroscopy and/or PXRD.

Differential Scanning Calorimetry

DSC was performed on a TA Instruments DSC Q10. Samples were placed in aluminum hermetic pans and sealed using a TA Instruments crimper. The temperature range was 20–300°C with a heating rate of 10°C/min.

RESULTS AND DISCUSSION

PXRD

PXRD is a particularly diagnostic way of differentiating between OCB polymorphs. Form I has characteristic peaks at $2\theta = 10.36, 12.10, \text{ and } 14.43^\circ$. Form II has a greater number of intense peaks than form I and can be distinguished by those at $2\theta = 11.82, 14.22, 18.20, 21.79, \text{ and } 24.60^\circ$. Form III is easily identifiable by the presence of a relatively low angle peak at $4.95^\circ 2\theta$ and other prominent peaks at $8.58, 11.98, 13.08, \text{ and } 19.78^\circ 2\theta$. Table 1 lists all the peaks and their relative intensities and Figure 2 presents the powder patterns.

Raman Spectroscopy

Although the Raman spectra of forms I and II are very similar, they possess several regions where they can be differentiated. The characteristic areas where all three polymorphs are easily discriminated from one another are 100–200, 700–800, 1100–1300, 1500–1700, and 2900–3400 cm⁻¹. For form I, peaks occur at 162.3, 776.7, 1154.3, 1262.4, 1270.6, and 1570.3 cm⁻¹. The corresponding peaks in Form II are 159.5, 778.0, 1152.9, 1259.5, 1272.0, and 1568.9 cm⁻¹. The spectrum for form III differs significantly from those of forms I and II, making Raman spectroscopy an efficient method for its identification. Form III has corresponding peaks at 155.4, 779.4, 1154.3, 1258.1, 1276.1, and 1594.9 cm⁻¹. Significant differences are also observed in the hydrogen bonding region between 2900 and 3600 cm⁻¹. Here it can be observed that forms I and II have a similar hydrogen bonding motif within their crystal structure (see below), whereas form III is different as evidenced by the missing peak around 3340 cm⁻¹. Forms I and II also each have a peak around 3470 cm⁻¹ that is

shifted to 3340 cm⁻¹ in the spectrum of form III. A complete list of peaks is shown in Table 2 and the spectra are presented in Figure 3.

Infrared Spectroscopy

The IR spectra reveal the structural similarities and disparities between the polymorphs. From the spectra it can be concluded that the hydrogen bonding scheme exhibited by forms I and II are very similar. Form III has a different hydrogen bonding motif. In forms I and II the hydrogen bonding motif can be attributed to peaks at about 3471 and 3345 cm⁻¹, while in form III a significant shift and the addition of one peak is observed (3303, 3353, and 3435 cm⁻¹). Other major differences occur in the carbonyl region that can be attributed to the hydrogen bonding present in the crystal structure. In this region the peak that is at 1734 cm⁻¹ in form I and 1733 cm⁻¹ in form II disappears entirely from the spectrum of form III. Another significant dissimilarity is between 900 and 1000 cm⁻¹. Form I has peaks at 923, 956, and 985 cm⁻¹, while form II has peaks at 922, 959, and 994 cm⁻¹ and form III has peaks at 922, 949, 968, and 997 cm⁻¹. The IR spectra are shown in Figure 4 and the peak data are tabulated in Table 3.

Thermomicroscopy/DSC

The behavior of oxcabazepine during heating was observed on a hot stage (5°C/min) under crossed polarizers. No transitions were observed in form I before it melted. The DSC curve of form I showed an endotherm (melt/decomposition) with an onset of 222.6°C at a rate of 10°C with an enthalpy of 9.64 kcal/mol. Thermomicroscopy proved that for all polymorphs melting and decomposition occurred simultaneously and this contributes to the large melting range measured. During decomposition the melt turns from colorless to yellow. The decomposition of oxcabazepine is dependent upon the crystal size, with smaller and more defective crystals decomposing before larger ones.

A transition between 118 and 150°C was observed during the heating of form II. The temperature of this transition is highly dependent on crystal size and quality, with smaller crystals having more defects observed to transform before larger more perfect crystals. The DSC thermogram for form II exhibited an endothermic transition centered at 127.6°C followed immediately by a broad exothermic transition centered at 165.8°C. The approximate enthalpy of the endotherm is 0.23 kcal/mol while that of the exotherm was 0.42 kcal/mol. The endotherm is somewhat less than the predicted energy difference of 0.61 kcal/mol separating calculated form I and the next most stable form of Cabeza et al.¹³ The ensuing melt/decomposition has an onset of 218.2°C with an enthalpy of 7.97 kcal/mol. In order to determine which modification form II transformed into during heating, a sample was heated to 160°C and held there for 5 min. The sample was allowed to cool to room temperature. Raman microscopy and PXRD was performed on the product, which proved to be form I.

Form III showed a small transition between 115 and 125°C. The melt occurs between 210 and 216°C. The thermogram of form III shows a large endothermic transition between 58 and 150°C with a melt/decomposition onset of 210.0°C. The enthalpy of the transition is 6.23 kcal/mol. To determine which polymorph form III had transformed into, a sample was heated to 150°C and held there for 5 min. Upon cooling to room temperature, the sample was found to be form I by Raman microscopy. In all of the polymorphs the melt was concomitant with decomposition. The DSC thermograms are presented in Figure 5.

Single Crystal X-Ray Diffraction

The crystal structure of form I was re-determined at low temperature and that of form II was solved for the first time (Tab. 4, Figs. 6 and 7). The molecular conformation in forms I and

II are similar. In form I, the azepine ring is in a twist-boat conformation. The angle between the planes defined by the benzene rings is 63°. The carboxamide group is extended above the rest of the molecule and participates in hydrogen bonding directed by the carbonyl of the carboxamide group on one molecule binding with the hydrogen of the NH₂ of next molecule creating infinite chains along the *b*-axis. The length of the hydrogen bond (O...N) is 2.77 Å. This differs dramatically from the anti-hydrogen bonded dimers observed in the four polymorphs of carbamazepine.⁶ The second hydrogen in the carboxamide functionality participates in N-H... π bonding with the benzene ring nearest to it. In addition to hydrogen bonding, C-H...O interactions are present between the carbonyl of the azepine ring and a hydrogen on a benzene ring of a neighboring molecule thus creating dimers and connecting adjacent hydrogen bonded chains. As pointed out previously,¹³ form I oxcarbazepine is isostructural to form I of dihydrocarbamazepine.

The experimental crystal structure of form II is very similar to the second lowest energy predicted structure from Cabeza et al.¹³ The molecular conformation of form II is related to form I. The benzene rings are planar and the angle between the planes is 65°. Consistent with the vibrational spectroscopy, the carboxamide group adopts a hydrogen bonding scheme similar to that in form I with infinite chains parallel to the *b*-axis. The hydrogen bond (O...N) distance is 2.75 Å. Unlike form I, the hydrogen of the amide that does not participate in strong hydrogen bonding forms N-H... π interactions. A major difference between the structures of form I and form II is the nature of the C-H...O interactions. Instead of the dimers observed in form I, the carbonyl of the azepine ring interacts with two separate molecules from an adjacent chain, thus linking two different chains.

The structure of form III was never determined due to the small size and poor quality of the crystals. Crystal size and quality were unable to be improved upon, despite adjusting crystallization parameters and attempting seeding. The packing motif observed in forms I and II of oxcarbazepine differs greatly from that seen in carbamazepine and some of its other derivatives. One major difference from carbamazepine is the asymmetry of azepine ring in OCB. This leads to a greater puckering of the azepine ring on one side of the molecule. This is thought to allow for the optimization of the hydrogen bonding in a chain motif.¹³ All three structures of dihydrocarbamazepine also exhibit an analogous hydrogen bonding scheme to OCB, as they all form hydrogen bonded chains.⁷⁻⁹ The hydrogen bonding in all four of the carbamazepine polymorphs favor an anti-dimer motif,⁶ whereas the structure of epoxycarbamazepine displays a twisted syndimer configuration.¹⁸ In contrast to carbamazepine, dihydrocarbamazepine form I is almost isostructural to OCB form I and exhibits a similar conformation of the azepine ring.¹³ Hydroxycarbamazepine, although similar to OCB and epoxycarbamazepine in the fact that it adds an additional functional group that can participate in hydrogen bonding, differs from these structures as it actually hydrogen bonds through the alcohol in addition the carboxamide functionality and thus forms neither anti-dimers nor chains.¹⁹

CONCLUSION

OCB is at minimum trimorphic and two of these forms are structurally characterized. While the crystal structure for form III remains elusive, the presence of this form has been confirmed through multiple methods. The packing motif for forms I and II differ from those observed in the polymorphs of carbamazepine. This shows that although the basic molecular structure of carbamazepine and oxcarbazepine are similar, the change from a double bond bridge to an alkyl carbonyl is significant in the packing of the molecules. Because oxcarbazepine, dihydrocarbamazepine, and carbamazepine are polymorphic, the basic backbone of the molecules should be considered a polymorphophore.¹⁴ Additional work is needed to verify all of the polymorphic forms reported in the patent literature.^{10,11}

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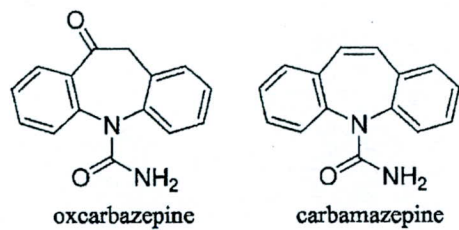


Figure 1.
Molecular structures of carbamazepine (left) and oxcarbazepine (right).

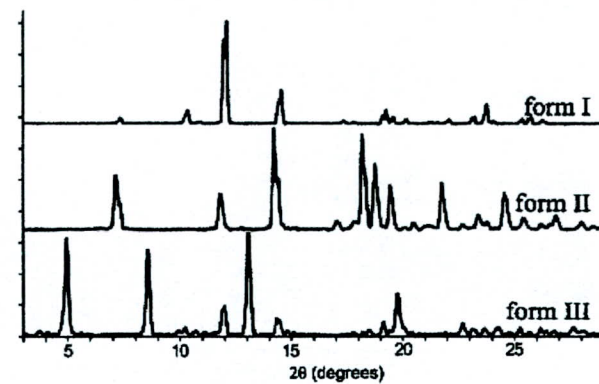


Figure 2.
PXRD patterns of the three polymorphs of OCB.

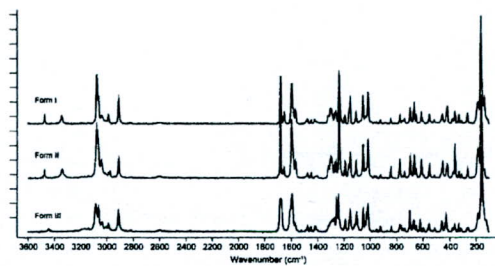


Figure 3.
Raman spectra of the three polymorphs of OCB.

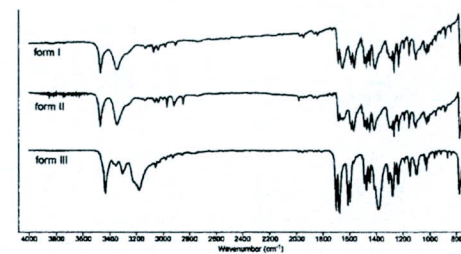


Figure 4.
Infrared spectra of forms I, II, and III of oxycarbazepine.

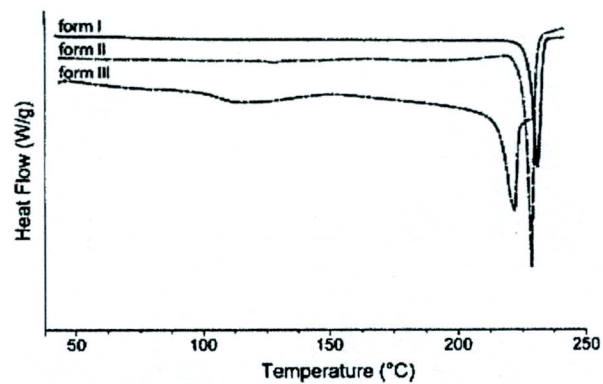


Figure 5.
DSC thermographs of the three polymorphs of OCB.

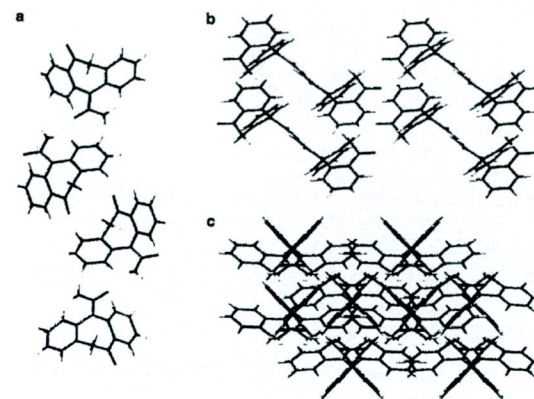


Figure 6.
Unit cell of form I. (a) View down *a*-axis, (b) view down *b*-axis, (c) view down *c*-axis.

57

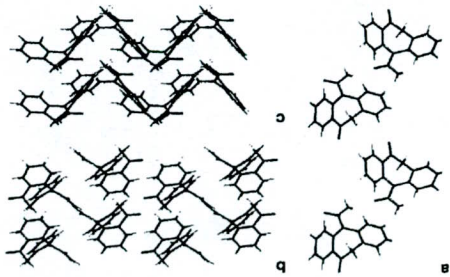


Figure 7. Unit cell of form II. (a) View down a-axis, (b) view down b-axis, (c) view down c-axis.

Table 1. Powder X-Ray Diffraction Peak Positions and Relative Intensities for the Polymorphs of OCB

2 θ (°)	Form I		Form II		Form III	
	Intensity (%)	2 θ (°)	Intensity (%)	2 θ (°)	Intensity (%)	2 θ (°)
7.36	5.0	7.14	55.2	4.95	100.0	
10.36	12.2	11.82	36.6	8.58	74.7	
11.96	81.0	14.22	100.0	11.98	27.3	
12.10	100.0	17.06	8.5	13.08	89.6	
14.43	21.2	17.87	7.3	14.34	15.6	
14.54	32.3	18.20	90.0	19.12	12.6	
17.39	2.8	18.76	60.1	19.78	34.1	
17.84	1.2	19.48	41.9			
19.10	7.7	20.11	1.9			
19.27	12.4	20.49	6.6			
19.60	6.6	21.18	3.5			
20.19	3.7	21.79	42.5			
21.28	1.9	22.69	4.2			
22.12	3.9	23.40	13.0			
23.13	4.7	23.81	6.4			
23.24	6.0	24.60	34.7			
23.78	17.2	25.42	10.3			
24.12	2.8	26.22	4.9			
25.36	4.1	26.87	13.2			
25.68	5.9	28.00	6.2			
25.76	6.3	28.54	2.0			
26.28	2.8	29.60	7.6			
29.88	5.2	32.90	2.8			
41.49	2.8	34.15	2.6			
	35.79		2.8			
	36.14		1.8			
	38.06		9.0			
	44.55		2.8			

57

Table 2

Summary of Raman Peak Positions for the Three Polymorphs of OCB

Raman Shift (cm ⁻¹)	Form I	Form II	Form III
100–200	3 peaks at 134.9, 162.3, and 185.9	2 peaks at 159.5 with shoulder at 134.9 and 184.2	2 peaks at 155.4 with shoulder at 124.0 and 184.2
200–300	1 peak at 266.3	1 peak at 263.5	2 peaks at 259.4 and 269.0
300–400	4 peaks at 310.1, 330.6, 362.1, and 378.5	3 peaks at 310.1, 329.2, and 360.7 with shoulder at 375.7	3 peaks at 314.2, 330.6, and 362.1 with shoulder at 377.1
400–500	2 peaks at 418.2 with shoulder at 425.0 and 453.7	2 peaks at 418.2 and 452.4	2 peaks at 429.1 and 457.8
500–600	2 peaks at 515.3 and 553.6	2 peaks at 513.9 and 552.2	3 peaks at 511.2, 555.0, and 598.8
600–700	4 peaks at 612.5 with shoulder at 598.8, 652.1, 668.6, and 698.7	4 peaks at 612.0 with shoulder at 601.0, 652.1, 667.2, and 697.3	3 peaks at 622.0, 650.8, and 671.3
700–800	3 peaks at 743.8, 753.4 with shoulder at 758.9, and 776.7	2 peaks at 743.8 and 778.0	5 peaks at 700.0, 742.4, 756.1, 767.1, and 779.4
800–900	2 peaks at 845.1 and 886.1	3 peaks at 846.4, 882.0, and	2 peaks at 842.3 and 872.4
900–1000	3 peaks at 924.4, 950.4, and 962.7	3 peaks at 923.1, 950.4, and 966.8	2 peaks at 923.1 and 950.4
1000–1100	2 peaks at 1016.1 with shoulders at 987.4, 1031.2, and 1055.8	3 peaks at 1014.7 with shoulder at 994.2, 1038.0, and 1055.8	3 peaks at 1016.1 (broad) with shoulder at 998.3, 1039.4, and 1053.1
1100–1200	3 peaks at 1107.8, 1154.3 with shoulder at 1163.8, and 1194.0	3 peaks at 1106.4, 1152.9 with shoulder at 1165.3, and 1192.6	3 peaks at 1103.7 with shoulder at 1117.3, 1154.3, and 1191.3
1200–1300	2 peaks at 1237.8 and 1262.4 with shoulder at 1270.6	3 peaks at 1237.8, 1259.7, and 1272.0	5 peaks at 1244.6, 1258.3, 1276.1, 1287.0, and 1298.0 (broad) with shoulders at 1306.5 and 1317.1
1300–1400	1 peak at 1303.5 (broad) with shoulder at 1324.0	1 peak at 1300.7 (broad) with shoulder at 1321.2	0 peaks
1400–1500	3 peaks at 1423.9 with shoulder at 1406.1, 1451.2, and 1477.2 with shoulder at 1490.9	3 peaks at 1406.1, 1451.2, and 1477.2 with shoulder at 1491.3	3 peaks at 1419.8, 1451.2, and 1477.2
1500–1600	2 peaks at 1570.3 and 1597.6 with shoulder at 1586.7	2 peaks at 1568.9 and 1597.6 with shoulder at 1587.4	3 peaks at 1523.8, 1571.6 and 1594.9 with shoulder at 16-07.2
1600–1700	2 peaks at 1653.7 and 1681.1	2 peaks at 1655.1 and 1681.1	2 peaks at 1645.5 and 1675.6 with shoulder at 1683.8
2500–2600		1 peak at 2599.3	1 peak at 2597.9
2800–2900	1 peak at 2820.9	1 peak at 2814.1	1 peak at 2819.6
2900–3000	2 peaks at 2909.9 and 2986.5	2 peaks at 2909.9 and 2975.5 with shoulder at 2986.5	2 peaks at 2911.2 and 2986.5
3000–3100	4 peaks at 3004.3, 3042.6 with shoulder at 3033.0, and 3076.8 with shoulder at 3063.1	4 peaks at 3005.6, 3018.0, 3042.6, and 3073.6 with shoulder at 3063.2	4 peaks at 3005.6, 3037.1 with shoulder at 3039.9, 3061.8, and 3085.0
3100–3200	1 peak at 3142.5	3 peaks at 3141.1, 3171.2, and 3193.1	3 peaks at 3141.1, 3168.5, and 3187.6
3300–3400	1 peak at 3345.0	1 peak at 3342.3	0 peaks
3400–3500	1 peak at 3473.6	1 peak at 3475.0	1 peak at 3440.8

Table 3

Summary of IR Peak Positions for the Three Polymorphs of OCB

Wavenumber (cm ⁻¹)	Form I	Form II	Form III
700–800	4 peaks at 743, 750, 758, and 771	4 peaks at 742, 751, 758, and 776	4 peaks at 740, 754, 767, and 778
800–900	2 peaks at 846 and 885 with shoulder at 876	2 peaks at 847 and 888 with shoulder at 880	2 peaks at 841 and 870
900–1000	3 peaks at 923, 956, and 985	3 peaks at 922, 959 with shoulder at 948, and 994	4 peaks at 922, 949, 968 with shoulder at 977, and 997
1000–1100	2 peaks at 1013 and 1027 with shoulder at 1035	2 peaks at 1012 and 1026 with shoulder at 1035	3 peaks at 1026 with shoulder at 1014, 1036, and 1052
1100–1200	3 peaks at 1104 with shoulder at 1074, 1154, and 1192	3 peaks at 1105 with shoulder at 1114, 1153, and 1190	1 peak at 1101 (broad) with shoulder at 1159
1200–1300	3 peaks at 1235, 1269, and 1287 with shoulder at 1304	4 peaks at 1235, 1257, 1270, and 1287 with shoulder at 1295	5 peaks at 1233, 1241, 1255, 1277, and 1283 with shoulder at 1293
1300–1400	1 peak at 1313 with shoulder at 1321	1 peak at 1304	2 peaks at 1309 and 1384 (broad) with shoulder at 1417
1400–1500	5 peaks at 1410 with shoulder at 1372, 1448, 1459, 1474, and 1489 with shoulder at 1505	4 peaks at 1418 with shoulder at 1373, 1451 with shoulder at 1458, 1474, and 1489 with shoulder at 1505	4 peaks at 1449, 1456, 1474, and 1489 with shoulder at 1505
1500–1600	3 peaks at 1564, 1586, and 1595	4 peaks at 1539, 1568, 1587, and 1595	4 peaks at 1540, 1560, 1568, and 1595
1600–1700	2 peaks at 1652 and 1686	2 peaks at 1652 with shoulder at 1646 and 1661 and 1687 with shoulder at 1680	3 peaks at 1612, 1641, and 1678
1700–1800	3 peaks at 1716, 1734, and 1771	3 peaks at 1716, 1733, and 1771	3 peaks at 1703, 1771, and 1791
1800–1900	2 peaks at 1843 and 1867	2 peaks at 1843 and 1873	3 peaks at 1813, 1848, and 1875
1900–2000	2 peaks at 1949 and 1974	3 peaks at 1928, 1952, and 1982	3 peaks at 1921, 1953, and 1986
2800–2900		1 peak at 2849	1 peak at 2855 with shoulder at 2871
2900–3000	3 peaks at 2907, 2949, and 2984	2 peaks at 2917 and 2971	1 peaks at 2956 and 2981
3000–3100	5 peaks at 3001, 3014, 3037, 3070, and 3099	4 peaks at 3002, 3036, 3062, and 3098	2 peaks at 3037 with shoulder at 3029 and 3056
3100–3200	2 peaks at 3131 and 3163	1 peak at 3136	1 peaks at 3180 with shoulder at 3219
3300–3400	1 peak at 3343 with shoulder at 3272	1 peak at 3346 with shoulder at 3272	2 peaks at 3303 and 3353 with shoulder at 3370
3400–3500	1 peak at 3470	1 peak at 3472	1 peak at 3435

Table 4

Cell Parameters for Forms I and II

	Form I	Form II
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1$
Unit cell		
<i>a</i>	5.20194(16) Å	5.1606(2) Å
<i>b</i>	9.2638(3) Å	9.4057(4) Å
<i>c</i>	24.7989(7) Å	12.5984(5) Å
α	90°	90°
β	95.234(2)°	92.720(3)°
γ	90°	90°
Volume	1190.07	610.825
Z	4	2
Density	1.408 g/cm ³	1.372 g/cm ³
Crystal size	0.20 × 0.07 × 0.06 mm	0.15 × 0.03 × 0.01 mm
Goodness of fit	1.017	1.040
Final <i>R</i> indices (obs data) [$I > 2\sigma(I)$]	0.0404	0.1280
Final <i>R</i> indices (all data)	0.0465	0.1954
w <i>R</i>	0.0905	0.3024

Polymorphism in Drugs and its Significance in Therapeutics

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A polymorph is a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of that compound in the solid state. Two polymorphs will be different in crystal structure but identical in the liquid and vapour states. Dynamic isomers will melt at different temperatures like polymorphs but give melts of different composition. Tautomers also behave in a confusingly similar manner like polymorphs. Polymorphism (a term which has a different meaning in pharmacogenetics) is the ability of any element or compound to crystallize as more than one distinct crystal species. Solubility, melting point, density, hardness, crystal shape, optical and electrical properties, vapour pressure, etc. vary with the polymorphic form. Polymorphism plays an important and significant role in the field of pharmaceuticals.

Some compounds may exhibit pseudopolymorphism by forming variously solvated crystals; each of these crystals, including the unsolvated form, may exhibit all the aspects of polymorphism. It is important to confine the term polymorphism to differences in packing, arrangement of atoms etc. of a single molecular species in the crystal lattice. The solvated (hydrated) crystals, or the amorphous forms, do not meet the above definition of polymorphism and hence can be termed pseudopolymorphs. By heat transfer, the polymorphic forms may transform to one another in the solid state and such a transformation is enantiotropic when the change is reversible, and monotropic when the change is irreversible. A knowledge of the behaviour of polymorphs supplements the utilization of the proper form of the drug substance to achieve the best performance.

There are some reviews¹⁻³ on the subject of polymorphism and its importance in pharmaceuticals. The work of Kuhnert-Brandstaetter², using thermomicroscopic methods, reported on the polymorphism of steroids, barbiturates and antihistamines, represents an intensive study of polymorphism in drugs. Haleblan and McCrone¹ reviewed the pharmaceutical applications of polymorphism and outlined crystal chemistry.

This article reviews the existence of polymorphism in pharmaceuticals — antibiotics, antibacterials, steroids, analgesics, anti-inflammatory agents and antipyretics, tranquillizers, hypnotics and sedatives and other drugs with reference to biopharmaceutics.

A number of techniques⁴, namely microscopy, hot-stage methods, infrared spectroscopy (IR), thermal methods, X-ray diffraction (XRD), proton magnetic resonance spectroscopy (pmr), nuclear magnetic resonance spectroscopy (nmr) and Raman spectroscopy, are also applied to identify the different polymorphic phases present in a drug. Using only one method for confirming the presence of polymorphs may be misleading, but a combination of methods provides a means for isolation and identification of each crystalline modification.

The existence and investigation methods of polymorphs in different categories of drugs are detailed below.

Antibiotics

After an extensive study of IR spectra of various forms, Austin *et al.*⁵ concluded that ampicillin crystallizes in two crystalline modifications, the anhydrate and trihydrate. Bellows *et al.*⁶, studying the polymorphism by Raman spectroscopy, stated that two anhydrous polymorphs and one trihydrate are distinguishable. Marsh and Weiss⁷ found a variation in the solubility of the anhydrous forms

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and the trihydrate from different manufacturers and attributed this to polymorphism. For cloxacillin sodium, differential thermal analysis (DTA)⁸ shows broad endothermic transitions with peak temperatures at 176°C and 193°C which indicate the existence of polymorphs in the drug.

The existence of two crystalline forms was reported for chlortetracycline hydrochloride⁹ based on the distinctly different IR and XRD patterns. For the base (chlortetracycline) crystallized from water or methanol, the XRD patterns are distinguishable.

For the polyene antibiotic, nystatin, three crystal forms¹⁰ were observed, which were identified by their IR spectra, thermal behaviour and XRD patterns. The *d* values (in Å) of the three intense peaks of XRD patterns in the order of decreasing intensity for the three forms are: from A - 4.32, 4.45, 6.34; Form B - 3.77, 4.52, 4.00; and Form C - 4.10, 20.0, 6.28. Porowska *et al.*¹¹ adopted a countercurrent distribution technique to establish the close chemical relationship between polyfungin and nystatin and showed that three forms of nystatin could be separated from two different solvent systems. Ghielmetti *et al.*¹² have shown that an occasional modification of isolation methods of mepartricin, nystatin or other polyene antibiotics may result in polymorphic modifications with differences in solubility and bioavailability. DTA of Rifampin¹³ shows an endotherm at 193°C corresponding to melting, immediately followed by an exotherm corresponding to the recrystallization of the melt, which then decomposes at about 240°C. This type of behaviour indicates the existence of polymorphic forms.

The other group of antibiotics is the cephalosporins. DTA of cephradine¹⁴ indicates pseudopolymorphism. Byrn *et al.*¹⁵ have established the existence of several pseudopolymorphs for sodium cephalosporin. All the forms are interconvertible by either loss or gain of water/solvent. For cephradine¹⁶, four polymorphs or rather pseudopolymorphs have been characterized by IR and XRD patterns. Cephradine has a tendency to form solvates; acetonitrile and ethyleneglycol solvates have been observed. The *d* values (in Å) of XRD patterns of four of the forms at which intense peaks are obtained are as follows:

cephradine, hydrated:	5.34, 4.32, 4.90
cephradine dihydrate:	3.55, 5.61, 2.92
cephradine monohydrate: (from acetonitrile-water)	9.92, 4.23, 8.83
cephradine monohydrate: (from methanol)	11.20, 4.74, 4.59

Table 1—*d* Values (Å) of Three Intense Peaks of the Solvated Forms of Cephalexin and Cephaloglycin

Solvated form	Cephalexin	Cephaloglycin
Dihydrate	13.00, 04.21, 05.80	16.80, 05.40, 07.15
Monohydrate	11.85, 05.39, 03.60	09.40, 04.69, 04.28 (anhydrate)
Diacetonitrilate	09.81, 04.19, 06.64	—
Formamidate	10.40, 04.38, 04.08	10.90, 04.60, 03.56
Methanolate	04.85, 04.65, 03.98	10.64, 04.69, 03.50 (Methanolate hydrate)
Acetonitrile hydrate	09.50, 04.76, 03.96	—

The existence of polymorphism was investigated in cephaloridine^{16(a)} by NMR, IR and XRD patterns and at least six different crystalline forms are reported. Sodium cefoxitin¹⁷ exists in several solvated and in a desolvated crystalline form. An amorphous form has also been identified. These polymorphs were distinguished by XRD patterns and also polarized light microscopy.

Pfeiffer *et al.*¹⁸ have shown that cephalexin and cephaloglycin occur in several solvated crystal forms and often in varying mixtures of these forms. These are termed as pseudopolymorphs and are characterized by XRD patterns; the *d* values (Å) of three intense peaks of each form are given in Table 1.

The existence and identify of these phases are also confirmed by their unique microscopic appearance and the solubility diagrams. For cephalosporin hydrates, interconversion of the forms is observed with variation in relative humidity.

Antibacterials

Differential scanning calorimetry (DSC) investigations have shown that sulphathiazole exists in two polymorphic forms¹⁹. Yang and Guillory²⁰ reported the presence of two polymorphs for sulphamethazine. For chloroquin phosphate²¹, DSC studies reveal the occurrence of two crystal forms with the first endotherm at 207.5°C and the other one at 226°C for a mixture of the two forms. The melting range of the usual form is 193-195°C. Both forms can be obtained from aqueous solution by selective crystallization. No polymorphs are reported for chloroquin base.

For clotrimazole, Borka and Valdimarsdóttir²² have reported two forms with distinct IR spectra, a stable one and a metastable one with 141-145°C and 106°C as melting points respectively. Lin and Guillory²³ have established four polymorphic modifications for sulphanilamide by DTA, DSC and IR studies.

Steroids

Dexamethasone²⁴ exists in two crystal forms A and B with distinct XRD patterns. Form B is the more usually prevalent one. Three polymorphic forms exist for estrone²⁵ depending on the mode of crystallization. Hot-stage microscopy²⁶ was performed on fluoxymesterone and the results indicate evidence of polymorphism. For testolactone, two modifications were observed on the Kofler hot-stage²⁷. The existence of two polymorphic forms for triamcinolone diacetate²⁸ was confirmed by DTA and XRD studies. These crystal forms in sterile aqueous suspensions were also observed by scanning electron microscopy²⁹. In triamcinolone acetonide also, XRD³⁰ plays a decisive role in differentiating the two existing polymorphs.

Analgesics, Antipyretics and Antiinflammatory Reagents

Schwartzmann³¹ stated that the different crystal habits in aspirin were caused by the solvents used for crystallization. Flufenamic acid exists in five different forms³² distinguishable by their melting points, IR spectra and DSC data. Monkhouse and Lach³³ reported two modifications for indomethacin characterized by melting point, IR and XRD data. The principal peaks for Form I occur at d values (Å) of 7.63, 5.21 and 4.08 and for Form II, at 10.05, 6.24 and 6.13. Solvated forms of the drug with benzene, *t*-butyl alcohol and other solvents are also observed³⁴. Ketoprofen exists in two crystal forms³⁵.

Two interconvertible crystal polymorphs of pentazocine hydrochloride⁴ are known with melting points of 218°C and 254°C. Phenylbutazone exists in three polymorphic forms according to Matsunaga *et al.*³⁶. In addition, two pseudopolymorphs from cyclohexane and isobutanol were found. The 2θ values of three peaks of XRD patterns³⁷ of the three polymorphs are: α : 8.7, 19.4, 6.9; β : 8.35, 20.2, 7.05 and δ : 7.9, 20.7, 7.05.

Sulindac³⁸ can exist in two nonsolvated enantiotropic crystal forms designated as Form I (mp 191°C) and Form II (mp 186°C). DTA, IR and XRD patterns distinguish the forms. Diflunisal exists in three nonsolvated crystal forms³⁹. The d values (Å) of the XRD patterns for the three forms at which peaks are observed are: Form I: 21.5, 6.7, 5.2; Form II: 6.1, 7.4, 5.4; and Form III: 5.8, 7.1, 5.3.

Tranquillizers

DSC investigations of benperidol⁴⁰ indicated three polymorphic forms. Form I has an endothermic peak at 177°C. Form II, which is obtained by recrystallization from isopropanol, melted at

169°C and Form III, obtained by recrystallization from *n*-heptane, melted at 154°C. The existence of polymorphic forms and a hydrated form in droperidol⁴¹ is partially demonstrated by a melting point determination. Azibi *et al.*⁴⁰ have shown that benperidol and droperidol form three pseudopolymorphic forms each. They form solvates, which are dehydrated forms and may exist in amorphous state also.

Hypnotics and Sedatives

DSC investigations show the existence of polymorphs for glutethimide⁴². Heroin⁴³ can exist in two polymorphic forms. Burger and Schulte⁴⁴ reported three modifications for mebroamate, which are distinguishable by thermal data, IR, XRD and dissolution studies. Lorazepam⁴⁵ forms solvates and other crystal forms.

Other Drugs

DTA and XRD data confirmed the existence of two polymorphic forms and an amorphous form for iopanoic acid⁴⁶. Vidler⁴⁷ has investigated the IR and X-ray crystallographic aspects of penicillamine and established the existence of two polymorphic forms. Levodopa⁴⁸ occurs as two modifications, which are characterized by the difference in unit cell parameters.

Two polymorphic forms, characterized by IR, XRD and DTA studies are reported for tolbutamide⁴⁹. Captopril⁵⁰ exists in two modifications with melting points of 86°C and 106°C with distinct IR, XRD and unit cell parameters. The d values (Å) of three lines of XRD pattern are: high melting form — 4.47, 3.16, 3.43; and low melting form — 4.02, 4.82, 10.15. Nabilone^{15,51} exists in at least four distinct forms depending upon the solvent and crystallization conditions and these are characterized by DTA and XRD studies.

Kuhnert-Brandstaetter and Wurian⁵² reported polymorphism in benactyzine hydrochloride, clenbuterol hydrochloride, diphenidol hydrochloride, dipyridamol and ethyl gallate. Through thermoanalytical investigations, Chauvet and Masse⁵³ have reported the existence of polymorphs in certain psychotherapeutic drugs such as chlorpromazine hydrochloride and fluphenazine dihydrochloride. Burger³ stated that the following drugs exhibit polymorphism, the number of polymorphs being given in parentheses: acetohexamide(3); chlorpropamide(5); methexamide(4); glymidine sodium(2); flufenamic acid(7); mefenamic acid(2); metazolin(3); pyrrithyldione(4); sulphapyridine(5). Some other drugs which exhibit polymorphism are given in Table 2.

Table 2—Drugs Which Exhibit Polymorphism and the Number of Forms

Sl. No.	Name of Drug	No. of polymorphs	Ref. No.
1	Flumetramide	5	54
2	Gvanoxyfen sulphate	5	
3	Cyheptamide	3	
4	Phenpromethamin hydrochloride	3	
5	Pipobroman	3	
6	Bolandiol dipropionate	3	55
7	Clominox	3	
8	Amisometradine	2	
9	Etafedrine hydrochloride	2	
10	Piribedil	2	
11	Anilamate	3	56
12	Dimethoxanate hydrochloride	2	
13	Diphenadione	2	
14	Maprotiline hydrochloride	3	
15	Nafoxidine hydrochloride	3	
16	Meperone	3	57
17	Benoxaprofen	2	15

Biopharmaceutic Aspects

The bioavailability of a drug is defined as its rate and extent of absorption and it is a measure of the amount of drug that a formulation makes available to the body. Other things being equal, a formulation that results in a significantly greater absorption of the drug would be preferred. Factors such as solubility, melting point, particle size, polymorphism, chemical form, permeability, gastro-intestinal degradation and rapid biotransformation of the drug significantly contribute to the availability characteristics. Sometimes, a change in the recrystallising solvent can result in a compound with different surface properties, which may alter the absorption properties of the compound.

Several reports suggest that solvated forms of a drug with organic solvents may dissolve in water much faster than the nonsolvated forms. Studies on humans indicate that the rate and extent of absorption of griseofulvin⁵⁸ were significantly increased after administration of the chloroform solvate compared to that observed after administration of the nonsolvated form of the drug. The microcrystalline form of griseofulvin produces higher blood levels⁵⁹. However, the micropulverization of a drug is not always advantageous. In the case of nitrofurantoin⁶⁰, the use of large crystals gave the required lower peak blood levels and thus reduced nausea and vomiting, which are the result of using the microcrystalline form of the drug.

Studies with sulphamethoxydiazine have indicated the occurrence of six polymorphs. Crystal-

line Form II is about twice as soluble as crystalline Form III. Studies in normal human subjects show that the rate and extent of absorption of the drug are 40% greater after administration of Form II than after administration of Form III⁶¹.

A prodrug is a chemical modification of an existing drug substance that, upon introduction to the appropriate biological system, reverts back to the parent compound by virtue of enzymatic or chemical liability. Prodrugs are designed to overcome one or more undesirable characteristics of the parent drug such as bitter taste, poor solubility and poor absorption. Certain prodrugs of ampicillin are active. Loo *et al.*⁶² have shown that the percentage of ampicillin absorbed from oral administration of pivampicillin (pivaloyloxymethyl ester of ampicillin) is more than that of the trihydrate and anhydrous forms of ampicillin¹.

The polymorphic forms of polyene antibiotics, mepartricin and nystatin have influence on toxicity¹². Marked differences in LD₅₀ values were found between various batches after intraperitoneal administration in suspension to mice. The samples obtained by different chemical treatments have different solubilities and bioavailabilities and hence variations in toxicity.

Physical processes such as heating, grinding or prolonged storage of the bioavailable polymorphic forms may result in nonbioavailable form. In the case of nabilone¹⁵, an effective way to prevent this conversion is to keep the drug forms dispersed in the water-soluble matrix of polyvinyl pyrrolidone (PVP).

Polymorphs have different compression behaviours. For any given mean compressive force, the tensile strengths⁶³ are as follows:

Sulphathiazole	Form II > Form I
Barbital	Form III > Form I > Form II
Aspirin	Form IV > Form I

The compression behaviours of chlorpropamide³ and meproamate⁶⁴ are also given. Compression also will result in transformations among the polymorphs. Caffeine, sulphabenzamide and maprotiline hydrochloride were subjected to trituration tests⁶⁵. The extent of transformation depended on the tablet, the pressure applied and the drug particle size.

Corrigan *et al.*⁶⁶ have shown that spray-drying of drugs like phenobarbitone and hydroflumethiazide can result in the formation of high energy drug polymorphic or amorphous phases, which are not normally obtained by conventional precipitation procedures.

Huettenrauch⁶⁷ has studied the application of activation theory to the tableting behaviour of polymorphic substances. Data on true density, melting point and tablet hardness have been interpreted. In general, hardness is inversely related to true density and melting point and lattice stability increases with increasing density.

Conclusion

Polymorphism plays a significant role in the field of pharmaceuticals — its existence in the first place in drugs and secondly in formulation additives and suppositories. It may be essential for a manufacturer to study these aspects in detail before arriving at the dosage forms.

SUMMARY

The existence of various crystalline modifications in compounds leads to the phenomenon of polymorphism, which is also noticed in some drugs. The possibility of formation of hydrated/solvated forms of drugs, which are termed as pseudopolymorphs, has opened new vistas in their therapeutics. The drugs are categorized into different groups, viz. antibiotics, antibacterials, steroids, analgesics, antiinflammatory drugs and antipyretics, tranquillizers, hypnotics and sedatives, psychotherapeutic and other drugs.

Various methods such as microscopy, hot-stage methods, IR, thermal methods, XRD, pmr spectroscopy, nmr and Raman spectroscopy are used to characterize the polymorphic forms. Detailed investigations by the above methods enrich the data on polymorphic modifications. X-ray diffraction studies on the samples can be considered as confirmative and conclusive evidence for the existence of the particular modification.

The polymorphic forms of about 80 drugs in the above categories are discussed. The review highlights the biopharmaceutical aspects of the polymorphic forms of the above drugs in their formulations. Factors such as solubility, melting point, particle size, chemical form, permeability and gastro-intestinal degradation of the drug significantly influence the bioavailability. The choice of the proper polymorph is a factor for proper physical/chemical stability of the formulation and for its therapeutic efficacy.

Udupa *et al.*⁶⁸ have studied the effect of polymorphic transformations and solubilizing agents on absorption patterns of certain drugs such as ibuprofen, tinidazole, lorazepam, salbutamol sulphate and amoxycillin sulphate. The solubilizing agents used are PVP-K 30, PEG-4000 and Tween-80.

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