REYES & REYES
A B O G A D O S

1 de 73
194

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No 06-042082 00004-0000
Fecha 2007 08 30 15 02 52 Dep 2020 NUECREACIONI
Tra 11 PATENTEIYII Eve 378 FASENACIONALI
Act 329 CTOINFORMACION Folios 73

SEÑOR
SUPERINTENDENTE DE INDUSTRIA Y COM
E S D

REF	**EXPEDIENTE 06-042-082**
TÍTULO SOLICITADO	**"MÉTODO PARA PREPARAR PARTICULAS SUBMICRÓNICAS DE PACLITAXEL"**
PUBLICACIÓN	**GACETA 573 de 28 Febrero 2007, NUMERO DE PUBLICACION 1120**
SOLICITANTE	**BAXTER INTERNATIONAL INC**
PRIORIDAD	**US 10/703 395 de 07/11/2003**
APODERADO	**EMILIO FERRERO**
TRÁMITE	**SUSTENTACION DE OPOSICIÓN**

JOSE LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional numero 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUIMICAS S A ,** dentro del tramite de la referencia en ejercicio de lo dispuesto por el articulo 42 inciso 2º de la Decision 486 de 2000, por medio del presente escrito, procedo a sustentar y complementar la oposicion presentada el 30 de mayo de 2007 en los siguientes terminos

I RATIFICACIÓN

Comedidamente me permito insistir en los argumentos planteados en la oposicion, los cuales demuestran que la solicitud de la referencia incumple los requisitos de patentabilidad establecidos por los articulos 14, 16 y 18 de la Decision 486 de 2000 y que por ello no merece recibir el beneficio patentario

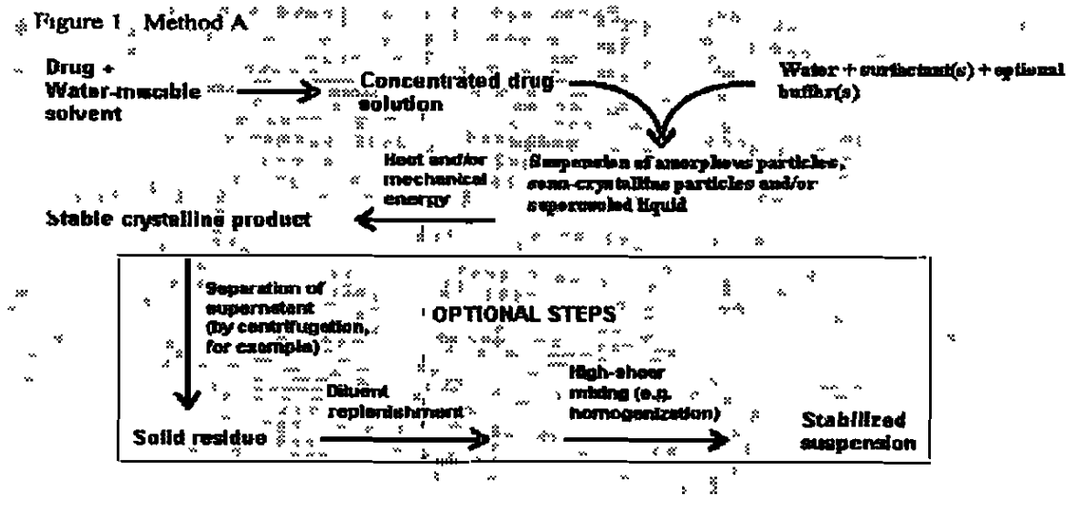
Me permito ratificar los argumentos expuestos, con el fin de justificar la razon por la cual debe negarse la solicitud que nos ocupa

- La publicacion internacional WO 99/059550 publicada el 25 de noviembre de 1999 y titulada **"NOVEL PARTICULATE FORMULATIONS"** revela procedimientos similares e involucra caracteristicas identicas en las composiciones y formas de obtencion de las particulas de paclitaxel reivindicadas por la solicitud colombiana en trámite, es decir, el procedimiento para obtener particulas submicronicas de paclitaxel, asi como las tecnicas y demas compuestos necesarios para llevar a acabo dicho proceso se encuentran revelados y suficientemente descritos dentro del estado de la tecnica, en consecuencia la novedad y el nivel inventivo de la solicitud colombiana en cuestion se encuentran afectados por obra de la anterioridad mencionada

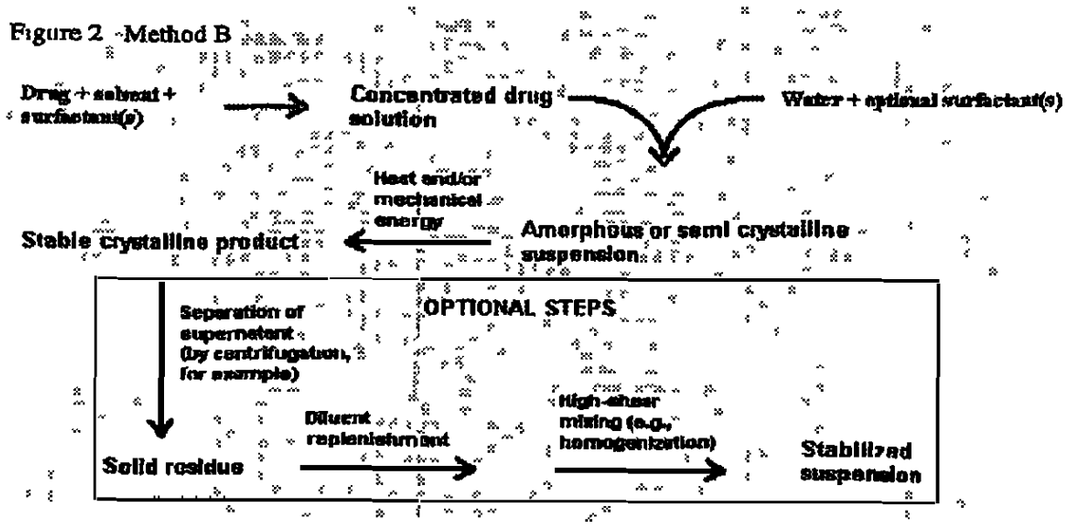
II COMPLEMENTO DE INFORMACIÓN

- La publicacion internacional WO/2002/055059 publicada el 18 de Julio de 2002 y titulada "METHOD FOR PREPARING SUBMICRON PARTICLE SUSPENSIONS" revela métodos o procesos para formar o preparar particulas de tamaño submicronico de un compuesto farmaceuticamente activo, donde la solubilidad es mayor en un primer solvente organico miscible en agua que en un segundo solvente que es acuoso estos procesos se pueden clasificar en tres categorias generales donde cada una comparte los pasos de 1) Disolver el compuesto organico farmaceuticamente activo en un primer solvente organico miscible en agua, para formar la primera solucion, 2) mezclar la primera solucion con el segundo solvente para precipitar el compuesto activo y crear la pre-suspension , y 3) adicionar energia a la presuspension en forma de high-shear mixing o calor para proporcionar una forma estable del compuesto organico teniendo los rangos de tamaño deseados

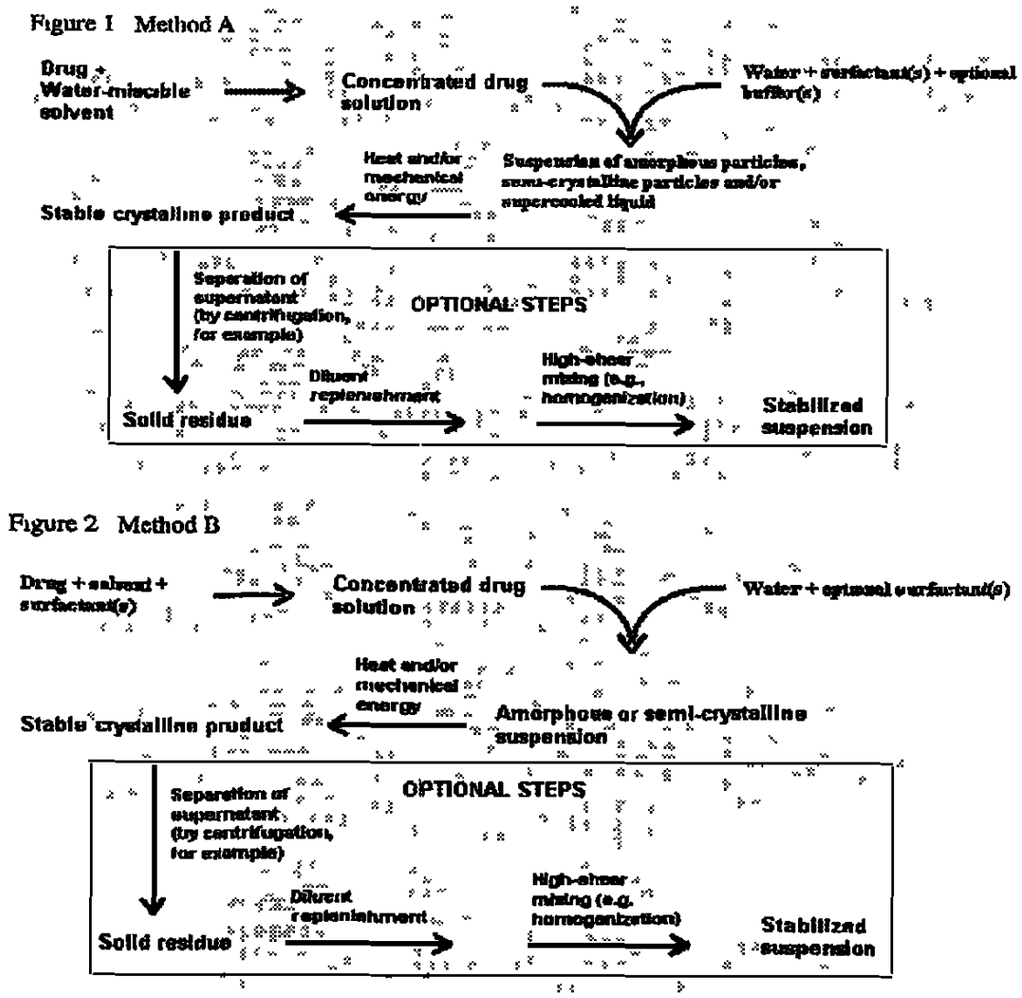
El primer metodo se caracteriza por



Y el segundo se caracteriza por



Si se observa con detenimiento lo revelado anteriormente y se compara con lo descrito en la publicacion internacional WO 05/046671 paginas 51 y 52 (equivalente a la solicitud colombiana en tramite)



Tenemos que en ambos documentos se emplean los mismos metodos de obtención de pequeñas particulas de manera tal que, los metodos propuestos en la solicitud colombiana en tramite, carece por completo de novedad y nivel inventivo

Para entrar en mas detalles es importante resaltar que lo revelado en la materia descriptiva de la anterioridad citada -WO 02/055059-, en especial lo relacionado con las ejemplos 1 a 18 (paginas 16 a 35) y figuras 3 a 17, es ***totalmente idéntico*** *a lo revelado en la parte descriptiva de la solicitud* internacional WO 05/046671 (equivalente a la solicitud colombiana en tramite), paginas 27 a 44 y figuras 3 a 17 ya que ellos describen

WO 02/055059	**WO 05/046671 (equivalente a la CO 06-042-082)**
(Pag 15) Example 1 Preparation of itraconazole suspension by use of Process Category 1 method A with homogenization	(Pag 26) Example 1 Preparation of itraconazole suspension by use of Process Category 1 method A with homogenization
(Pag 17) Example 2 Preparation of itraconazole suspension by use of Process Category 1 method A with ultrasonication	(Pág 28) Example 2 Preparation of itraconazole suspension by use of Process Category 1 method A with ultrasonication
(Pag 19) Example 3 Preparation of itraconazole suspension by use of Process Category 1, method B with homogenization	(Pag 29) Example 3 Preparation of itraconazole suspension by use of Process Category 1 method B with homogenization
(Pag 19 y 20) Example 4 Preparation of itraconazole suspension by use of Process Category 1 method B with ultrasonication	(Pag 30) Example 4 Preparation of itraconazole suspensión by use of Process Category 1 method B with ultrasonication
(Pag 20) Example 5 Preparation of itraconazole suspension (1%) with 0 75% Solutol® HR (PEG-660 12-hydroxystearate) Process Category 2 Method B	(Pag 31) Example 5 Preparation of itraconazole suspension (1%) with 0 75% Solutol® HR (PEG-660 12-hydroxystearate) Process Category 2, Method B
(Pag 22) Example 6 Preparation of carbamazepine by use of Process Category 3 Method A with homogenization	(Pag 32) Example 6 Preparation of carbamazepine by use of Process Category 3 Method A with homogenization
(Pag 22) Example 7 Preparation of 1% carbamazepine suspension with 0 125% Solutol® by use of Process Category 3 Method B with homogenization	(Pág 32) Example 7 Preparation of 1% carbamazepine suspension with 0 125% Solutol® by use of Process Category 3 Method B with homogenization
(Pag 23) Example 8 Preparation of 1% carbamazepine suspension with 0 06% sodium glycodeoxycholate and 0 06% poloxamer 188 by use of Process Category 3 Method B with homogenization	(Pag 33) Example 8 Preparation of 1% carbamazepine suspension with 0 06% sodium glycodeoxycholate and 0 06% poloxamer 188 by use of Process Category 3 Method B with homogenization
(Pag 25) Example 9 Preparation of 1 6% (w/v) prednisolone suspension with 0 05% sodium deoxycholate and 3% N-methyl-2-pyrrolidinone Process Category 3 Method B	(Pag 35) Example 9 Preparation of 1 6% (w/v) prednisolone suspension with 0 05% sodium deoxycholate and 3% N-methyl-2-pyrrolidinone Process Category 3 Method B
(Pag 28) Example 10 Preparation of prednisolone suspension by use of Process Category 3 Method A with homogenization	(Pag 36) Example 10 Preparation of prednisolone suspension by use of Process Category 3 Method A with homogenization

WO 02/055059	WO 05/046671 (equivalente a la CO 06-042-082)
(Pag 29) Example 11 Preparation of nabumetone suspension by use of Process Category 3, Method B with homogenization	(Pag 37) Example 11 Preparation of nabumetone suspension by use of Process Category 3 Method B with homogenization
(Pag 29) Example 12 Preparation of nabumetone suspension by use of Process Category 3 Method B with homogenization and the use of Solutol® HS 15 as the surfactant Replacement of supernatant liquid with a phsopholipid medium	(Pag 37) Example 12 Preparation of nabumetone suspension by use of Process Category 3, Method B with homogenization and the use of Solutol® HS 15 as the surfactant Replacement of supernatant liquid with a phsopholipid medium
(Pag 29) Example 13 Preparation of itraconazole suspension with poloxamer with particles of a mean diameter of aproximately 220nm	(Pag 38) Example 13 Preparation of itraconazole suspension with poloxamer with particles of a mean diameter of aproximately 220nm
(Pag 31) Example 14 Preparation of 1% itraconazole nanosuspension with hydroxyethylstarch	(Pag 38) Example 14 Preparation of 1% itraconazole nanosuspension with hydroxyethylstarch
(Pag 31) Example 15 Prophetic examole of Method A using HES	(Pag 40) Example 15 Prophetic example of Method A using HES
(Pag 31 y 32) Example 16 Seeding during Homogenization to convert a Mixture of Polymorphs to the more stable Polymorph	(Pag 40) Example 16 Seeding during Homogenization to convert a Mixture of Polymorphs to the more stable Polymorph
(Pag 33) Example 17 Seeding during Precipitation to preferentially Form a Stable Polymorph	(Pag 42) Example 17 Seeding during Precipitation to preferentially Form a Stable Polymorph
(Pag 33) Example 18 Polymorph control by seeding the drug concentrate	(Pag 42) Example 18 Polymorph control by seeding the drug concentrate
(Pag 17) Figure 3 Amorphous particles prior to homogenization (example 1)	(Pag 52) Figure 3 Amorphous particles prior to homogenization (example 1)
(Pag 17) Figure 4 Particles after annealing by homogenization	(Pag 53) Figure 4 Particles after annealing by homogenization
(Pag 21) Figure 5 X-Ray diffractogram of *microprecipitated* itraconazole with polyethylene glycol-660-12-hydroxystearate and after homogenization (example 5)	(Pag 54) Figure 5 X-Ray diffractogram of *microprecipitated* itraconazole with polyethylene glycol-660-12-hydroxystearate and after homogenization (example 5)
(Pag 24) Figure 6 Carbamazepine crystals before homogenization (example 6)	(Pag 55) Figure 6 Carbamazepine crystals before homogenization (example 6)
(Pag 24) Figure 7 Carbamazepine microparticulate after homogenization (avestin C-50) (example 6)	(Pag 56) Figure 7 Carbamazepine microparticulate after homogenization (avestin C-50) (example 6)
(Pág 26) Figure 8 Diagram of Microprecipitation process for Prednisolone (Examples 9-12)	(Pag 57) Figure 8 Diagram of Microprecipitation process for Prednisolone (Examples 9-12)

WO 02/055059	WO 05/046671 (equivalente a la CO 06-042-082)
(Pag 27) Figure 9 Photomicrograph of Prednisolone suspension before homogenization	(Pag 58) Figure 9 Photomicrograph of Prednisolone suspension before homogenization
(Pag 27) Figure 10 Photomicrograph of Prednisolone suspension after homogenization	(Pag 59) Figure 10 Photomicrograph of Prednisolone suspension after homogenization
(Pag 31) Figure 11 Comparison of size distributions of nanosuspensions (this invention) and comercial fat emulsion (example 13)	(Pag 60) Figure 11 Comparison of size distributions of nanosuspensions (this invention) and comercial fat emulsion (example 13)
(Pag 34) Figure 12 X-ray powder diffraction patterns for raw material itraconazole (top) and SMP-2-PRE (bottom) The raw material has been shifted upward for clarity (example 16)	(Pág 61) Figure 12 X-ray powder diffraction patterns for raw material itraconazole (top) and SMP-2-PRE (bottom) The raw material has been shifted upward for clarity (example 16)
(Pag 35) Figure 13a DSC trace for raw material itraconazole (example 16)	(Pág 62) Figure 13a DSC trace for raw material itraconazole (example 16)
(Pag 35) Figure 13b DSC trace for SMP-2-PRE (example 16)	(Pag 62) Figure 13b DSC trace for SMP-2-PRE (example 16)
(Pag 36) Figure 14 DSC trace for SMP-2-PRE showing the melt of the less stable polymorph upon heating to 160°C a recrystallization event upon cooling, and the subsequent meeting of the more stable polymorph upon reheating to 180°C (example 16)	(Pág 63) Figure 14 DSC trace for SMP-2-PRE showing the melt of the less stable polymorph upon heating to 160°C a recrystallization event upon cooling, and the subsequent meeting of the more stable polymorph upon reheating to 180°C (example 16)
(Pag 36) Figure 15 Comparison of SMP-2-PRE samples alter homogenization Solid line= sample seeded with raw material itraconazole Dashed line= unseeded simple The solid line has been shifted by 1 W/g for clarity (example 16)	(Pág 64) Figure 15 Comparison of SMP-2-PRE samples alter homogenization Solid line= sample seeded with raw material itraconazole Dashed line= unseeded simple The solid line has been shifted by 1 W/g for clarity (example 16)
(Pag 37) Figure 16 Effect of seeding during precipation Dashed line= unseeded sample Solid line= sample seeded with raw material itraconazole The unseeded trace (dashed line) has been shifted upward by 1 5 W/g for clarity (example 17)	(Pag 65) Figure 16 Effect of seeding during precipation Dashed line= unseeded sample Solid line= sample seeded with raw material itraconazole The unseeded trace (dashed line) has been shifted upward by 1 5 W/g for clarity (example 17)
(Pag 37) Figure 17 Effect of seeding the drug concentrate through aging Top X-ray diffraction pattern is for crystals prepared from fresh drug concentrate and is consistent with the stable polymorph (see Figure 12 top) Bottom pattern is for crystals prepared from aged (seeded) drug concentrate and is consistent with the	(Pag 66) Figure 17 Effect of seeding the drug concentrate through aging Top X-ray diffraction pattern is for crystals prepared from fresh drug concentrate and is consistent with the stable polymorph (see Figure 12 top) Bottom pattern is for crystals prepared from aged (seeded) drug concentrate and is consistent with the metastable

WO 02/055059	WO 05/046671 (equivalente a la CO 06-042-082)
metastable polymorph (see Figure 12 bottom) The top pattern has been shifted upward for clarity (example 18)	polymorph (see Figure 12 bottom) The top pattern has been shifted upward for clarity (example 18)

Adicionalmente es importante llamar la atención de este Despacho en cuanto a que si bien es cierto en la anterioridad nombrada tantas veces no se hace referencia explicita a Paclitaxel, no lo es menos que los metodos y/o procesos descritos para preparar particulas submicronicas –y que como se menciono son iguales a los referidos en la solicitud colombiana en tramite- son aplicables a compuestos farmacéuticamente activos pertenecientes a varios grupos tales como, **pero no limitados a** antihiperlipidemicos, antimicrobianos antibacteriales como sulfadiazina, antifungicos como itraconazol, antiinflamatorios no esferoidales como indometacina, antihipercolesterolémicos como probucol, compuestos esteroidales como dexametasona inmunosupresores como ciclosporina A tacrolimus, y mofetil micofenolato

Si adicional a esto tenemos presente tambien que

The present invention provides a method for preparing submicron sized particles of an organic compound the solubility of which is greater in a water-miscible first solvent than in a second solvent which is aqueous The process includes the steps of (i) dissolving the organic compound in the water-miscible first solvent to form a solution, the first solvent being selected from the group consisting of N methyl-2-pyrrolidinone, 2 pyrrolidone, dimethyl sulfoxide, dimethylacetamide, lactic acid, medianol,

( )

dicaprylate, propylene glycol dicaprate, propylene glycol laurate, (ii) mixing the solution with the second solvent to define a pre-suspension; and (iii) adding energy to the pre-suspension to form particles having an average effective particle size of less than about 2 μm.

( )

In Method A (see Figure 1) the organic compound ("drug") is first dissolved in the first solvent to create a first solution The organic compound can be added from about 0 1% (w/v) to about 50% (w/v) depending on the solubility of the organic compound in the first solvent Heating of the concentrate from about 30°C to about 100°C may be necessary to ensure total dissolution of the compound in the first solvent

( )

deoxycholate, etc) Suitable cationic surfactants include but are not limited to quaternary ammonium compounds such as benzalkonium chloride cetyltrimethylammonium bromide, laurildimethylbenzylammonium chloride, acyl carnitine hydrochlorides or alkyl pyridinium halides As anionic surfactants, phospholipids may be used Suitable phospholipids include, for example phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid lysophospholipids, egg or soybean phospholipid or a combination thereof The phospholipid may be salted or desalted hydrogenated or partially hydrogenated or natural semisynthetic or synthetic

( )

Suitable nonionic surfactants include polyoxyethylene fatty alcohol ethers (Macrogol and Brij), polyoxyethylene sorbitan fatty acid esters (Polysorbates) polyoxyethylene fatty acid esters (Myrj), sorbitan esters (Span), glycerol monostearate, polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-

( )

Surface active biological molecules include such molecules as albumin casein, heparin, hirudin or other appropriate proteins

Y que ademas se reivindica

1 A method for preparing submicron sized particles of an organic compound, the solubility of which is greater in a water-miscible first solvent than in a second solvent which is aqueous, the process comprising the steps of

(i) dissolving the organic compound in the water-miscible first solvent to form a solution the first solvent being selected from the group consisting of N-methyl 2-pyrrolidinone, 2-pyrrolidone, dimethyl sulfoxide, dimethylacetamide, lactic acid, methanol ethanol isopropanol, 3-pentanol, n propanol, glycerol, butylene glycol, ethylene glycol, propylene glycol, mono and diacylated monoglycerides, dimethyl isosorbide, acetone, dimethylformamide 1 4-dioxane, polyethylene glycol, polyethylene glycol esters, polyethylene glycol sorbitans polyethylene glycol monoalkyl ethers, polypropylene glycol, polypropylene alginate, PPG-10 butanediol, PPG 10 methyl glucose ether, PPG-20 methyl glucose ether, PPG 15 stearyl ether, propylene glycol dicaprylate, propylene glycol dicaprate, propylene glycol laurate,

(ii) mixing the solution with the second solvent to define a pre-suspension, and

(iii) adding energy to the pre-suspension to form particles having an average effective particle size of less than about 2 μm

8 The method of claim 7 wherein the anionic surfactant is a copolymer of oxyethylene and oxypropylene.

9 The method of claim 8 wherein the copolymer of oxyethylene and oxypropylene is a block copolymer

36 The method of claim 31 wherein the first solvent is N-methyl-2 pyrrolidinone

39 The method of claim 31 further comprising the step of mixing into the solution a sixth surface modifier selected from the group consisting of anionic surfactants, cationic surfactants, nonionic surfactants and surface active biological modifiers.

41 The method of claim 40 wherein the phospholipid is selected from natural phospholipids and synthetic phospholipids

52 The method of claim 50 wherein the phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, lysophospholipids, egg phospholipid and soybean phospholipid

65 The method of claim 60 wherein the particles of the presuspension have an average effective particle size of less than about 400 nm.

Podemos ver claramente que en el estado de arte se describe minuciosamente el procedimiento (Método A y B) que sustenta la invencion de la solicitud colombiana en tramite, adicionalmente, especifica el uso de fosfolipidos naturales o sinteticos, surfactantes anionicos, cationicos, no ionicos y modificadores biologicos de superficie activa, copolimeros de oxietileno y oxipropileno, la utilizacion de N-metil-pirrolidona como el primer solvente miscible en agua, el rango de temperatura utilizado durante el proceso y el rango de tamaño de particula obtenido

- La publicacion internacional WO/2003/045330 publicada el 05 de Junio de 2003 y titulada "MATERIALS AND METHODS FOR MAKING IMPROVED MICELLE COMPOSITIONS" revela

compound, or delivery of the compound in an inactive conformation. According to one aspect of the present invention, polyethylene-glycol (PEG) is covalently conjugated to DSPE and used to form polymeric micelles which are then passively loaded with VIP. The PEG-DSPE forms micelles with a hydrophobic core consisting of distearoyl phosphatidylethanolamine (DSPE) fatty acid chains which is surrounded by a hydrophilic "shell" formed by the PEG polymer

( )

In another aspect, the invention provides methods for preparing a biologically active sterically stabilized crystalline product comprising one or more biologically active compounds which are insoluble in an aqueous solution, said method comprising the steps of: a) dissolving in an organic solvent said biologically active compound(s) and one or more lipids wherein at least one lipid is conjugated to a water soluble polymer; b) removing the organic solvent to leave a dry film; c) hydrating the dry film with an aqueous solution, and d) forming a sterically stabilized crystalline product. As used herein and in subsequently described crystalline products

( )

Methods of the invention for producing sterically stabilized crystalline products are amenable to the use of any compound that is insoluble in an aqueous solution Preferred insoluble compounds include, but are not limited to, progesterone, testosterone, estrogen, prednisolone, prednisone, 2,3 mercaptopropanol, amphotericin B, betulinic acid, camptothecin, diazepam, nystatin, propofol, cyclosporin A doxorubicin, and paclitaxel (Taxol®); and tetramethyl NDGA In methods of the

( )

In one embodiment, medicaments of the invention include micelle compositions or crystalline compounds wherein the water soluble polymer is polyethylene glycol (PEG) In another embodiment, medicaments of the invention include use of micelles having an average diameter of less than about 25 nm In still another embodiment, medicaments of the invention include micelle compositions or crystalline compounds wherein the combination of lipids consists of distearoyl-phosphatidylethanolamine covalently bonded to PEG (PEG-DSPE)

( )

or synthetic lipids with polymeric headgroups The most preferred polymer of the invention is PEG at a molecular weight between 1000 and 5000. Preferred lipids for producing micelles according to the invention include distearoyl-phosphatidylethanolamine covalently bonded to PEG (PEG-DSPE) alone or in further combination with phosphatidylcholine (PC), and phosphatidylglycerol (PG) in further combination with cholesterol (Chol) and/or calmodulin

( )

Methods of the invention for preparation of sterically stabilized micelle products or sterically stabilized crystalline products can be carried using various techniques In one aspect micelle components are mixed in an organic solvent and the solvent is removed using either evaporation or lyophilization. Removal of the

( )

Sterically stabilized crystalline products of the invention are particularly useful for administration of anti-cancer agents For example, crystalline products of the invention comprising paclitaxel (Taxol®) as the insoluble compound and VIP as the targeting agent can be targeted to breast cancer cells which are known

( )

Como vemos, en el estado de la técnica claramente se evidencia el uso de los procedimientos de cristalización que sustenta la invencion de la solicitud colombiana en tramite, especificamente en caracteristicas tales como el uso de un fosfolipido conjugado con un polimero hidrofilico o soluble en agua, la remocion de la fase liquida a traves de evaporación y liofilización, y finalmente la aplicacion de este metodo en particulas de compuestos insolubles en agua mas especificamente en paclitaxel

- La patente americana US 5 916 596 publicada el 29 de junio de 1999 y titulada 'PROTEIN STABILIZED PHARMACOLOGICALLY ACTIVE AGENTS, METHODS FOR THE PREPARATION THEREOF AND METHODS FOR THE USE THEREOF" revela

> In accordance with the present invention, there are provided compositions and methods useful for the in vivo delivery of substantially water insoluble pharmacologically active agents (such as the anticancer drug paclitaxel) in which the pharmacologically active agent is delivered in the form of suspended particles coated with protein (which acts as a stabilizing agent) In particular protein and pharmacologically active agent in a biocompatible dispersing medium are subjected to high shear in the absence of any conventional surfactants, and also in the absence of any polymeric core material for the particles The procedure yields particles with a diameter of less than about 1 micron The use of specific composition and preparation conditions (e g , addition of a polar solvent to the organic phase), and careful selection of the proper organic phase and phase fraction enables the reproducible production of unusually small nanoparticles of less than 200 nm diameter, which can be sterile-filtered The particulate system produced according to the invention can be converted into a redispersible dry powder comprising nanoparticles of water-insoluble drug coated with a protein, and free protein to which molecules of the pharmacological agent are bound This results in a unique delivery system in which part of the pharmacologically active agent is readily bioavailable (in the form of molecules bound to the protein) and part of the agent is present within particles without any polymeric matrix therein
>
> ( )
>
> In accordance with the present invention, we have discovered that substantially water insoluble pharmacologically active agents can be delivered in the form of microparticles or nanoparticles that are suitable for parenteral administration in aqueous suspension This mode of delivery
>
> ( )
>
> Thus, in accordance with the present invention, there are provided methods for the formation of nanoparticles of pharmacologically active agents by a solvent evaporation technique from an oil in-water emulsion prepared under conditions of high shear forces (e g , sonication, high pressure homogenization, or the like) without the use of any conventional surfactants, and without the use of any polymeric core material to form the matrix of the nanoparticle Instead, proteins (e g , human serum albumin) are employed as a stabilizing agent
>
> ( )
>
> The invention further provides a method for the reproducible formation of unusually small nanoparticles (less than 200 nm diameter), which can be sterile-filtered through a 0.22 micron filter This is achieved by addition of a water

( )

In accordance with the present invention, there are also provided submicron particles in powder form, which can easily be reconstituted in water or saline The powder is obtained after removal of water by lyophilization Human serum albumin serves as the structural component of invention nanoparticles, and also as a cryoprotectant and reconstitution aid The preparation of particles filterable through a 0 22 micron filter according to the invention method as described herein, followed by drying or lyophilization, produces a sterile solid formulation useful for intravenous injection

Ademas reivindica

6 A method according to claim 4 wherein said pharmaceutically active agent is an antineoplastic selected from adriamycin, cyclophosphamide, actinomycin, bleomycin duanorubicin, doxorubicin, epirubicin, mitomycin, methotrexate, fluorouracil carboplatin, carmustine (BCNU), methyl-CCNU, cisplatin, etoposide, interferon, camptothecin and derivatives thereof, phenesterine, paclitaxel and derivatives thereof, taxotere and derivatives thereof, vinblastine, vincristine, tamoxifen, etoposide or piposulfan

27 A method according to claim 26 further comprising removing said organic phase from said mixture

28 A method according to claim 26 further comprising filtering said mixture through a 0 22 micron filter

29 A method according to claim 26 further comprising removing said aqueous phase from said mixture

30 A method according to claim 26 wherein said high shear conditions produce amorphous particles, crystalline particles, or a mixture thereof

Lo anterior permite demostrar claramente que en el estado de arte ya era ampliamente conocido el uso del procedimiento que sustenta la invencion de la solicitud Colombiana en tramite, especificamente lo que respecta al uso de paclitaxel como principio activo, uso de modificadores biologicos de superficie activa, los rangos de tamaño de particular obtenidos, la esterilizacion del compuesto nanoparticulado por filtracion la remocion de la fase liquida para la obtencion de un polvo seco de particulas a traves de evaporacion, liofilizacion o filtracion de alta presion y la formulacion adecuada para administracion parenteral

En consecuencia ruego se analicen los argumentos expuestos con el rigor y detenimiento habituales, y que en consecuencia el Despacho decrete, ademas de la negación de la solicitud en comento, el archivo del expediente que lo contiene

III ANEXOS

- La publicación internacional WO/2002/055059 publicada el 18 de Julio de 2002 y titulada "METHOD FOR PREPARING SUBMICRON PARTICLE SUSPENSIONS "
- La publicacion internacional WO/2003/045330 publicada el 05 de Junio de 2003 y titulada "MATERIALS AND METHODS FOR MAKING IMPROVED MICELLE COMPOSITIONS" Paginas 17, 20 21, 26, 30 y 36
- La patente americana US 5,916,596 publicada el 29 de junio de 1999 y titulada "PROTEIN STABILIZED PHARMACOLOGICALLY ACTIVE AGENTS, METHODS FOR THE PREPARATION THEREOF AND METHODS FOR THE USE THEREOF" Columnas 5, 6 y capitulo reivindicatorio

Señor Superintendente

JOSE LUIS REYES VILLAMIZAR
C C 79 152 473 de Usaquen
T P 44655 del C S de la J

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 July 2002 (18 07 2002)

PCT

(10) International Publication Number
WO 02/055059 A2

(51) International Patent Classification[7] A61K 9/10
31/496

(21) International Application Number PCT/US01/49737

(22) International Filing Date
20 December 2001 (20 12 2001)

(25) Filing Language English

(26) Publication Language English

(30) Priority Data

60/258 160	22 December 2000 (22 12 2000)	US
09/874 799	5 June 2001 (05 06 2001)	US
09/874 637	5 June 2001 (05 06 2001)	US
09/874,499	5 June 2001 (05 06 2001)	US
09/953 979	17 September 2001 (17 09 2001)	US
10/035 821	19 October 2001 (19 10 2001)	US
10/021 692	12 December 2001 (12 12 2001)	US

(71) Applicant BAXTER INTERNATIONAL INC [US/US] One Baxter Parkway Deerfield II 60015 (US)

(72) Inventors KIPP James E 609 Hermosa Avenue Wauconda II 60084 (US) WONG Joseph Chung, Tak 36141 N Bridlewood Avenue Gurnee II 60031 (US) DOTY Mark J 610 Kenilworth Grayslake, II 60030 (US) REBBECK, Christine, L. 525 Eagle Ridge Lane Algonquin II 60102 (US) BRYNJELSEN Sean 4 Walnut Grove Ct Lake In The Hills II 60156 (US) WERLING Jane 605 S Highland Arlington Heights IL 60005 (US) SRIRAM Rajaram 3014 Knollwood Glenview II 60025 (US)

(74) Agent BUONAIUTO, Mark J. Baxter International Inc One Baxter Parkway Deerfield II 60015 (US)

(81) Designated States *(national)* AE AG AI AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PH PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

(84) Designated States *(regional)* European patent (AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR)

Published
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations refer to the "Guidance Notes on Codes and Abbreviations appearing at the beginning of each regular issue of the PCT Gazette

WO 02/055059 A2

(54) Title METHOD FOR PREPARING SUBMICRON PARTICLE SUSPENSIONS

(57) Abstract The present invention provides a method for preparing a suspension of a pharmaceutically active compound the solubility of which is greater in a water miscible first organic solvent than in a second solvent which is aqueous The process includes the steps of (i) dissolving a first quantity of the pharmaceutically active compound in the water miscible first organic solvent to form a first solution (ii) mixing the first solution with the second solvent to precipitate the pharmaceutically active compound and (iii) seeding the first solution or the second solvent or the presuspension

METHOD FOR PREPARING SUBMICRON PARTICLE SUSPENSIONS

DESCRIPTION

CROSS-REFERENCE TO RELATED APPLICATION

Not Applicable

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Not Applicable

BACKGROUND OF THE INVENTION

Technical Field

The present invention relates to the preparation of a pharmaceutically active compound More particularly the invention relates to the manufacture of nanosuspensions of the pharmaceutically active compound for parenteral or oral delivery

Background Art

There is an ever increasing number of pharmaceutical drugs being formulated that are poorly soluble or insoluble in aqueous solutions Such drugs provide challenges to delivering them in an injectable form such as through parenteral administration Drugs that are insoluble in water can have significant benefits when formulated as a stable suspension of sub micron particles Accurate control of particle size is essential for safe and efficacious use of these formulations Particles must be less than seven microns in diameter to safely pass through capillaries without causing emboli (Allen et al , 1987, Davis and Taube 1978, Schroeder et al 1978, Yokel et al , 1981)

One approach to delivering an insoluble drug is disclosed in United States Patent No 2,745,785 This patent discloses a method for preparing crystals of penicillin G suitable for parenteral administration The method includes the step of recrystallizing the penicillin G from a formamide solution by adding water to reduce the solubility of the penicillin G The '785 Patent further provides that the penicillin G particles can be coated with wetting agents such as lecithin, or emulsifiers surface active and defoaming agents, or partial higher fatty acid esters of sorbitan or polyoxyalkyklene derivatives thereof, or aryl alkyl polyether alcohols or salts thereof The '785 patent further discloses micronizing the penicillin G with an air blast under pressure to form crystals ranging from about 5 to 20 microns

Another approach is disclosed in United States Patent No 5,118,528 which discloses a process for preparing nanoparticles The process includes the steps of (1) preparing a liquid phase of a

substance in a solvent or a mixture of solvents to which may be added one or more surfactants (2) preparing a second liquid phase of a non-solvent or a mixture of non-solvents, the non-solvent is miscible with the solvent or mixture of solvents for the substance, (3) adding together the solutions of (1) and (2) with stirring and (4) removing of unwanted solvents to produce a colloidal suspension of nanoparticles The '528 Patent discloses that it produces particles of the substance smaller than 500 nm without the supply of energy In particular the '528 Patent states that it is undesirable to use high energy equipment such as sonicators and homogenizers

United States Patent No 4,826,689 discloses a method for making uniformly sized particles from water-insoluble drugs or other organic compounds First, a suitable solid organic compound is dissolved in an organic solvent, and the solution can be diluted with a non-solvent. Then, an aqueous precipitating liquid is infused, precipitating non-aggregated particles with substantially uniform mean diameter The particles are then separated from the organic solvent. Depending on the organic compound and the desired particle size, the parameters of temperature ratio of non-solvent to organic solvent, infusion rate, stir rate, and volume can be varied according to the invention The '689 Patent discloses this process forms a drug in a metastable state which is thermodynamically unstable and which eventually converts to a more stable crystalline state The '689 Patent discloses trapping the drug in a metastable state in which the free energy lies between that of the starting drug solution and the stable crystalline form The '689 Patent discloses utilizing crystallization inhibitors (e g polyvinylpyrrolidinone) and surface-active agents (e g , poly(oxyethylene)-co-oxypropylene)) to render the precipitate stable enough to be isolated by centrifugation membrane filtration or reverse osmosis

In U S Patent Nos 5,091,188, 5,091,187 and 4,725,442 which disclose (a) either coating small drug particles with natural or synthetic phospholipids or (b) dissolving the drug in a suitable lipophilic carrier and forming an emulsion stabilized with natural or semisynthetic phospholipids One of the disadvantages of these approaches is they rely on the quality of the raw material of the drug and do not disclose steps of changing the morphology of the raw material to render the material in a friable, more easily processed form

Another approach to providing insoluble drugs for parenteral delivery is disclosed in U S Patent No 5,145,684 The '684 Patent discloses the wet milling of an insoluble drug in the presence of a surface modifier to provide a drug particle having an average effective particle size of less than 400 nm. The '684 Patent emphasizes the desirability of not using any solvents in its process The '684 Patent discloses the surface modifier is adsorbed on the surface of the drug particle in an amount sufficient to prevent agglomeration into larger particles

Yet another attempt to provide insoluble drugs for parenteral delivery is disclosed in U S Patent Nos 5,922,355 The '355 Patent discloses providing submicron sized particles of insoluble drugs using a combination of surface modifiers and a phospholipid followed by particle size reduction using techniques such as sonication homogenization, milling microfluidization, precipitation or

recrystallization There is no disclosure in the '355 Patent of changing process conditions to make crystals in a more friable form.

United States Patent No 5,780,062 discloses a method of preparing small particles of insoluble drugs by (1) dissolving the drug in a water miscible first solvent (2) preparing a second solution of a polymer and an amphiphile in an aqueous second solvent in which the drug is substantially insoluble whereby a polymer/amphiphile complex is formed and (3) mixing the solutions from the first and second steps to precipitate an aggregate of the drug and polymer/amphiphile complex

United States Patent No 5 858 410 discloses a pharmaceutical nanosuspension suitable for parenteral administration The '410 patent discloses subjecting at least one solid therapeutically active compound dispersed in a solvent to high pressure homogenization in a piston-gap homogenizer to form particles having an average diameter determined by photon correlation spectroscopy (PCS) of 10 nm to 1000 nm, the proportion of particles larger than 5 μm in the total population being less than 0 1% (number distribution determined with a Coulter counter) without prior conversion into a melt, wherein the active compound is solid at room temperature and is insoluble, only sparingly soluble or moderately soluble in water, aqueous media and/or organic solvents The Examples in the '410 Patent disclose jet milling prior to homogenization

United States Patent No 4,997,454 discloses a method for making uniformly sized particles from solid compounds The method of the '454 Patent includes the steps of dissolving the solid compound in a suitable solvent followed by infusing precipitating liquid thereby precipitating non-aggregated particles with substantially uniform mean diameter The particles are then separated from the solvent. The '454 Patent discourages forming particles in a crystalline state because during the precipitating procedure the crystal can dissolve and recrystallize thereby broadening the particle size distribution range The 454 Patent encourages during the precipitating procedure to trap the particles in a metastable particle state

United States Patent No 5 605,785 discloses a process for forming nanoamorphous dispersions of photographically useful compounds The process of forming nanoamorphous dispersions includes any known process of emulsification that produces a disperse phase having amorphous particulates

SUMMARY OF THE INVENTION

The present invention provides a method for preparing submicron sized particles of an organic compound, the solubility of which is greater in a water-miscible first solvent than in a second solvent which is aqueous The process includes the steps of (1) dissolving the organic compound in the water-miscible first solvent to form a solution, the first solvent being selected from the group consisting of N methyl-2-pyrrolidinone 2-pyrrolidone, dimethyl sulfoxide dimethylacetamide, lactic acid, methanol ethanol, isopropanol, 3-pentanol, n propanol, glycerol butylene glycol, ethylene glycol propylene glycol mono- and diacylated monoglycerides, dimethyl isosorbide, acetone, dimethylformamide, 1,4-

dioxane polyethylene glycol polyethylene glycol esters, polyethylene glycol sorbitans, polyethylene glycol monoalkyl ethers, polypropylene glycol, polypropylene alginate, PPG-10 butanediol, PPG-10 methyl glucose ether, PPG-20 methyl glucose ether, PPG 15 stearyl ether, propylene glycol dicaprylate propylene glycol dicaprate propylene glycol laurate (ii) mixing the solution with the second solvent to define a pre-suspension, and (iii) adding energy to the pre-suspension to form particles having an average effective particle size of less than about 2 μm.

A method for preparing submicron sized particles of an organic compound the solubility of which is greater in a water-miscible first solvent than in a second solvent which is aqueous the process comprising the steps of (i) dissolving the organic compound in the water-miscible first solvent to form a solution, the first solvent being selected from the group consisting of N-methyl-2-pyrrolidinone, 2-pyrrolidone, dimethyl sulfoxide, dimethylacetamide, lactic acid, methanol, ethanol, isopropanol, 3-pentanol, n-propanol, glycerol, butylene glycol, ethylene glycol, propylene glycol mono- and diacylated monoglycerides, dimethyl isosorbide, acetone, dimethylformamide, 1,4-dioxane,ethyl acetate propyl acetate, polyethylene glycol, polyethylene glycol esters, polyethylene glycol sorbitans polyethylene glycol monoalkyl ethers, polypropylene glycol polypropylene alginate, PPG-10 butanediol, PPG-10 methyl glucose ether, PPG-20 methyl glucose ether, PPG 15 stearyl ether, propylene glycol dicaprylate, propylene glycol dicaprate, propylene glycol laurate, (ii) mixing the solution with the second solvent to define a pre-suspension wherein the organic compound is in an amorphous form, a semicrystalline form or in a supercooled liquid form as determined by DSC and having an average effective particle size and (iii) annealing the pre suspension to form particles having essentially the same average effective particle size of the pre-suspension and in a more stable form

The present invention further provides a method for preparing submicron sized particles of an organic compound, the solubility of which is greater in a water miscible first solvent than in a second solvent which is aqueous The process includes the steps of (i) dissolving the organic compound in the water-miscible first solvent to form a solution, the first solvent being selected from the group consisting of N-methyl-2-pyrrolidinone, 2-pyrrolidone, dimethyl sulfoxide, dimethylacetamide, lactic acid, methanol, ethanol, isopropanol, 3-pentanol, n-propanol, glycerol butylene glycol, ethylene glycol, propylene glycol, mono- and diacylated monoglycerides, dimethyl isosorbide acetone dimethylformamide, 1,4-dioxane, polyethylene glycol, polyethylene glycol esters, polyethylene glycol sorbitans, polyethylene glycol monoalkyl ethers, polypropylene glycol, polypropylene alginate, PPG-10 butanediol PPG-10 methyl glucose ether, PPG-20 methyl glucose ether, PPG-15 stearyl ether, propylene glycol dicaprylate, propylene glycol dicaprate, propylene glycol laurate, (ii) mixing the solution with the second solvent to define a pre-suspension of particles in a friable form, and (iii) adding energy to the pre-suspension to form particles having an average

effective particle size of less than about 2 μm

The present invention also provides a method for preparing a suspension of a pharmaceutically-active compound the solubility of which is greater in a water miscible first organic solvent than in a second solvent which is aqueous The process includes the steps of (i) dissolving a first quantity of the pharmaceutically-active compound in the water miscible first organic solvent to form a first solution (ii) mixing the first solution with the second solvent to precipitate the pharmaceutically-active compound and (iii) seeding the first solution or the second solvent or the presuspension The method further includes the step of forming a desired polymorph of the pharmaceutically active compound In a preferred form of the invention the step of seeding includes the step of adding a seed compound to the first solution to the second solvent and/or to the presuspension

The present invention further provides a method for preparing a suspension of a pharmaceutically-active compound the solubility of which is greater in a water miscible first organic solvent than in a second solvent which is aqueous The method includes the steps of (i) adding a quantity of the pharmaceutically-active compound to the first organic solvent to create a supersaturated solution, (ii) aging the supersaturated solution to form detectable crystals to create a seeding mixture and (iii) mixing the seeding mixture with the second solvent to precipitate the pharmaceutically-active compound to create a presuspension

BRIEF DESCRIPTION OF THE DRAWINGS

Not Applicable The Drawings have been incorporated into the text

DETAILED DESCRIPTION OF THE INVENTION

The present invention is susceptible of embodiments in many different forms Preferred embodiments of the invention are disclosed with the understanding that the present disclosure is to be considered as exemplifications of the principles of the invention and are not intended to limit the broad aspects of the invention to the embodiments illustrated

The present invention provides methods or processes for forming particles of an organic compound having an average effective particle size suitable for parenteral administration and, in a most preferred form of the invention, is less than about 2 μm The present invention is also suitable for providing particles of the organic compound in a form suitable for oral administration Particles sizes for oral dosage forms can be in excess of 2 μm and typically less than about 7μm However, the particles can exceed 7μm provided that the particles have sufficient bioavalability and other characteristics of an oral dosage form Oral dosage forms include tablets, capsules, caplets soft and hard gel capsules or other delivery vehicle for delivering a drug by oral administration

The processes can be separated into three general categories Each of the categories of processes share the steps of (1) dissolving an organic compound in a water miscible first organic solvent to create a first solution (2) mixing the first solution with a second solvent of water to precipitate the organic compound to create a pre-suspension and (3) adding energy to the presuspension in the form of high-shear mixing or heat to provide a stable form of the organic compound having the desired size ranges defined above

The three categories of processes are distinguished based upon the physical properties of the organic compound as determined through x-ray diffraction studies differential scanning calorimetry (DSC) studies or other suitable study conducted prior to the energy-addition step and after the energy-addition step In the first process category, prior to the energy-addition step the organic compound in the presuspension takes an amorphous form, a semi-crystalline form or a supercooled liquid form and has an average effective particle size After the energy-addition step the organic compound is in a crystalline form having an average effective particle size essentially the same as that of the presuspension (i e , from less than about 2μm)

In the second process category prior to the energy-addition step the organic compound is in a crystalline form and has an average effective particle size After the energy-addition step the organic compound is in a crystalline form having essentially the same average effective particle size as prior to the energy-addition step but the crystals after the energy-addition step are less likely to aggregate

The lower tendency of the organic compound to aggregate is observed by laser dynamic light scattering and light microscopy

In the third process category, prior to the energy-addition step the organic compound is in a crystalline form that is friable and has an average effective particle size. What is meant by the term "friable" is that the particles are fragile and are more easily broken down into smaller particles After the energy-addition step the organic compound is in a crystalline form having an average effective particle size smaller than the crystals of the pre-suspension By taking the steps necessary to place the organic compound in a crystalline form that is friable, the subsequent energy-addition step can be carried out more quickly and efficiently when compared to an organic compound in a less friable crystalline morphology

The energy-addition step can be carried out in any fashion wherein the pre-suspension is exposed to cavitation, shearing or impact forces In one preferred form of the invention, the energy-addition step is an annealing step Annealing is defined in this invention as the process of converting matter that is thermodynamically unstable into a more stable form by single or repeated application of energy (direct heat or mechanical stress), followed by thermal relaxation This lowering of energy may be achieved by conversion of the solid form from a less ordered to a more

ordered lattice structure Alternatively this stabilization may occur by a reordering of the surfactant molecules at the solid-liquid interface

These three process categories will be discussed separately below It should be understood however that the process conditions such as choice of surfactants or combination of surfactants, amount of surfactant used, temperature of reaction, rate of mixing of solutions, rate of precipitation and the like can be selected to allow for any drug to be processed under any one of the categories discussed next

The first process category, as well as the second and third process categories can be further divided into two subcategories Method A, and B shown diagramatically below

Figure 1 Method A

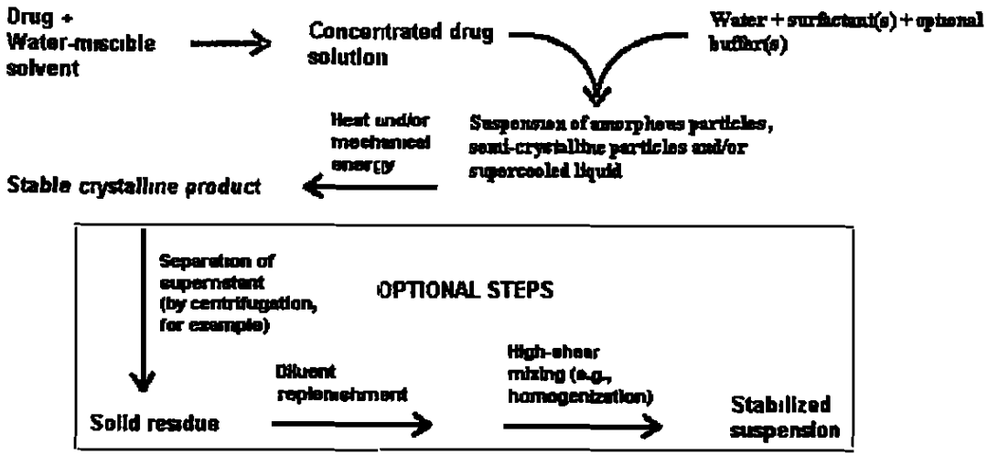
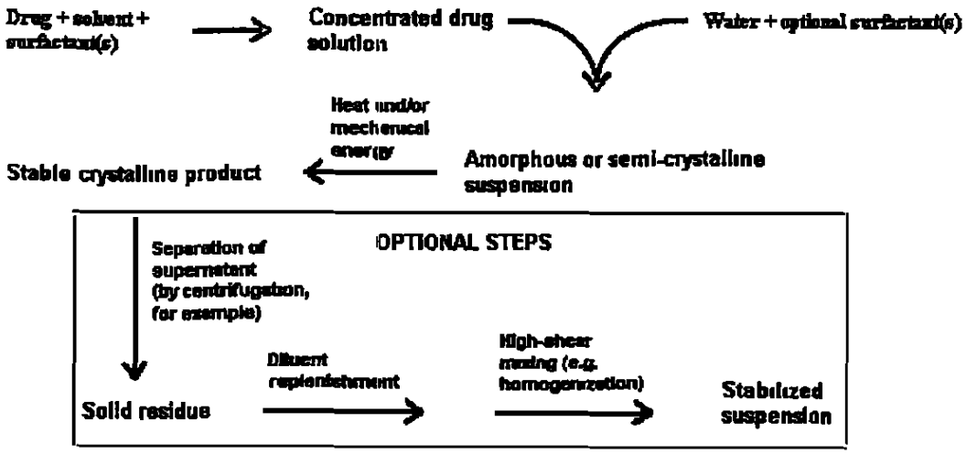


Figure 2 Method B



An organic compound for use in the process of this invention is any organic chemical entity whose solubility decreases from one solvent to another This organic compound might be a pharmaceutically active compound from various groups such as but not limited to antihyperlipidemics, antimicrobials, e g antibacterials such as sulfadiazine, antifungals such as itraconazole, non-steroidal anti-inflammatory drugs, e g , indomethacin antihypercholesteremic agents, e g probucol, and steroidal compounds e g dexamethasone immunosuppresants, e g cyclosporin A, tacrolimus, and mycophenolate mofetil Or the organic compound might be from the group used as adjuvants or excipients in pharmaceutical preparations and cosmetics, such as, but not limited to preservatives e g propylparaben

The first solvent according to the present invention is a solvent or mixture of solvents in which the organic compound of interest is relatively soluble and which is miscible with the second solvent Examples of such solvents include, but are not limited to polyvinylpyrrolidone, N-methyl-2 pyrrolidinone (also called N-methyl-2 pyrrolidone), 2-pyrrolidone, dimethyl sulfoxide dimethylacetamide, lactic acid, methanol, ethanol, isopropanol, 3-pentanol, n-propanol, glycerol butylene glycol (butanediol), ethylene glycol, propylene glycol, mono- and diacylated monoglycerides (such as glyceryl caprylate), dimethyl isosorbide, acetone, dimethylformamide 1,4-dioxane, polyethylene glycol (for example PEG 4, PEG-8, PEG-9 PEG-12 PEG-14 PEG-16 PEG 120, PEG-75, PEG-150, polyethylene glycol esters (examples such as PEG-4 dilaurate, PEG-20 dilaurate, PEG-6 isostearate PEG-8 palmitostearate, PEG-150 palmitostearate) polyethylene glycol sorbitans (such as PEG-20 sorbitan isostearate), polyethylene glycol monoalkyl ethers (examples such as PEG-3 dimethyl ether, PEG-4 dimethyl ether), polypropylene glycol (PPG), polypropylene alginate PPG 10 butanediol PPG-10 methyl glucose ether PPG-20 methyl glucose ether, PPG-15 stearyl ether, propylene glycol dicaprylate/dicaprate, propylene glycol laurate

Method A

In Method A (see Figure 1) the organic compound ("drug") is first dissolved in the first solvent to create a first solution The organic compound can be added from about 0 1% (w/v) to about 50% (w/v) depending on the solubility of the organic compound in the first solvent. Heating of the concentrate from about 30°C to about 100°C may be necessary to ensure total dissolution of the compound in the first solvent.

A second aqueous solution is provided with one or more optional surface modifiers such as an anionic surfactant, a cationic surfactant, a nonionic surfactant or a biological surface active molecule added thereto Suitable anionic surfactants include but are not limited to potassium laurate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl inosine, phosphatidylserine phosphatidic acid and their salts, glyceryl esters, sodium

carboxymethylcellulose, cholic acid and other bile acids (e g cholic acid deoxycholic acid glycocholic acid, taurocholic acid glycodeoxycholic acid), and salts thereof (e g , sodium deoxycholate, etc) Suitable cationic surfactants include but are not limited to quaternary ammonium compounds such as benzalkonium chloride cetyltrimethylammonium bromide lauryldimethylbenzylammonium chloride, acyl carnitine hydrochlorides or alkyl pyridinium halides As anionic surfactants, phospholipids may be used Suitable phospholipids include, for example phosphatidylcholine phosphatidylethanolamine phosphatidylserine phosphatidylinositol phosphatidylglycerol phosphatidic acid, lysophospholipids, egg or soybean phospholipid or a combination thereof The phospholipid may be salted or desalted hydrogenated or partially hydrogenated or natural semisynthetic or synthetic

Suitable nonionic surfactants include polyoxyethylene fatty alcohol ethers (Macrogol and Brij) polyoxyethylene sorbitan fatty acid esters (Polysorbates), polyoxyethylene fatty acid esters (Myrj), sorbitan esters (Span) glycerol monostearate, polyethylene glycols polypropylene glycols cetyl alcohol, cetostearyl alcohol stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers (poloxomers) polaxamines, methylcellulose hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, polysaccharides including starch and starch derivatives such as hydroxyethylstarch (HES) polyvinyl alcohol, and polyvinylpyrrolidone In a preferred form of the invention the nonionic surfactant is a polyoxyethylene and polyoxypropylene copolymer and preferably a block copolymer of propylene glycol and ethylene glycol Such polymers are sold under the tradename POLOXAMER also sometimes referred to as PLURONIC®, and sold by several suppliers including Spectrum Chemical and Ruger Among polyoxyethylene fatty acid esters is included those having short alkyl chains One example of such a surfactant is SOLUTOL® HS 15 polyethylene-660-hydroxystearate manufactured by BASF Aktiengesellschaft.

Surface active biological molecules include such molecules as albumin casein, heparin, hirudin or other appropriate proteins

It may also be desirable to add a pH adjusting agent to the second solution such as sodium hydroxide hydrochloric acid, tris buffer or citrate, acetate, lactate, meglumine, or the like The second solution should have a pH within the range of from about 3 to about 11

For oral dosage forms one or more of the following excipients may be utilized gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e g , macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters e g the commercially available Tweens TM polyethylene glycols, polyoxyethylene stearates, colloidol

silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium methylcellulose hydroxyethylcellulose, hydroxypropylcellulose hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP) Most of these excipients are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press 1986 The surface modifiers are commercially available and/or can be prepared by techniques known in the art Two or more surface modifiers can be used in combination

In a preferred form of the invention the method for preparing submicron sized particles of an organic compound includes the steps of adding the first solution to the second solution The addition rate is dependent on the batch size and precipitation kinetics for the organic compound Typically, for a small-scale laboratory process (preparation of 1 liter), the addition rate is from about 0 05 cc per minute to about 10 cc per minute During the addition, the solutions should be under constant agitation It has been observed using light microscopy that amorphous particles semi-crystalline solids, or a supercooled liquid are formed to create a pre-suspension The method further includes the step of subjecting the pre-suspension to an annealing step to convert the amorphous particles, supercooled liquid or semicrystalline solid to a crystalline more stable solid state The resulting particles will have an average effective particles size as measured by dynamic light scattering methods (e g , photocorrelation spectroscopy laser diffraction, low-angle laser light scattering (LALLS), medium-angle laser light scattering (MALLS) light obscuration methods (Coulter method, for example), rheology, or microscopy (light or electron) within the ranges set forth above)

The energy-addition step involves adding energy through sonication homogenization counter current flow homogenization, microfluidization, or other methods of providing impact, shear or cavitation forces The sample may be cooled or heated during this stage. In one preferred form of the invention the annealing step is effected by a piston gap homogenizer such as the one sold by Avestin Inc under the product designation EmulsiFlex-C160 In another preferred form of the invention, the annealing may be accomplished by ultrasonication using an ultrasonic processor such as the Vibra-Cell Ultrasonic Processor (600W), manufactured by Sonics and Materials, Inc In yet another preferred form of the invention, the annealing may be accomplished by use of an emulsification apparatus as described in U S Patent No 5 720 551 which is incorporated herein by reference and made a part hereof

Depending upon the rate of annealing, it may be desirable to adjust the temperature of the processed sample to within the range of from approximately –30°C to 30°C. Alternatively, in order

to effect a desired phase change in the processed solid it may also be necessary to heat the pre suspension to a temperature within the range of from about 30°C to about 100°C during the annealing step

Method B

Method B differs from Method A in the following respects The first difference is a surfactant or combination of surfactants is added to the first solution The surfactants may be selected from the groups of anionic nonionic and cationinc surfactants set forth above

Comparative Example of Method A and Method B and USPN 5 780 062

United States Patent No 5,780 062 discloses a process for preparing small particles of an organic compound by first dissolving the compound in a suitable water miscible first solvent A second solution is prepared by dissolving a polymer and an amphiphile in aqueous solution The first solution is then added to the second solution to form a precipitate that consists of the organic compound and a polymer-amphiphile complex The '062 Patent does not disclose utilizing the energy-addition step of this invention in Methods A and B Lack of stability is typically evidenced by rapid aggregation and particle growth In some instances, amorphous particles recrystallize as large crystals Adding energy to the pre suspension in the manner disclosed above typically affords particles that show decreased rates of particle aggregation and growth, as well as the absence of recrystallization upon product storage

Methods A and B are further distinguished from the process of the '062 patent by the absence of a step of forming a polymer-amphiphile complex prior to precipitation In Method A such a complex cannot be formed as no polymer is added to the diluent (aqueous) phase In Method B the surfactant, which may also act as an amphiphile, or polymer, is dissolved with the organic compound in the first solvent This precludes the formation of any amphiphile-polymer complexes prior to precipitation In the 062 Patent, successful precipitation of small particles relies upon the formation of an amphiphile polymer complex prior to precipitation. The 062 Patent discloses the amphiphile-polymer complex forms aggregates in the aqueous second solution The 062 Patent explains the hydrophobic organic compound interacts with the amphiphile-polymer complex thereby reducing solubility of these aggregates and causing precipitation In the present invention it has been demonstrated that the inclusion of the surfactant or polymer in the first solvent (Method B) leads, upon subsequent addition to second solvent, to formation of a more uniform, finer particulate than is afforded by the process outlined by the 062 Patent.

To this end, two formulations were prepared and analyzed Each of the formulations have two solutions a concentrate and an aqueous diluent, which are mixed together and then sonicated. The concentrate in each formulation has an organic compound (itraconazole), a water miscible

solvent (N-methyl-2-pyrrolidinone or NMP) and possibly a polymer (poloxamer 188) The aqueous diluent has water a tris buffer and possibly a polymer (poloxamer 188) and/or a surfactant (sodium deoxycholate) The average particle diameter of the organic particle is measured prior to sonication and after sonication

The first formulation A has as the concentrate itraconazole and NMP The aqueous diluent includes water, poloxamer 188, tris buffer and sodium deoxycholate Thus the aqueous diluent includes a polymer (poloxamer 188), and an amphiphile (sodium deoxycholate) which may form a polymer/amphiphile complex and, therefore, is in accordance with the disclosure of the '062 Patent (However, again the '062 Patent does not disclose an energy addition step)

The second formulation B has as the concentrate itraconazole, NMP and poloxamer 188 The aqueous diluent includes water, tris buffer and sodium deoxycholate This formulation is made in accordance with the present invention Since the aqueous diluent does not contain a combination of a polymer (poloxamer) and an amphiphile (sodium deoxycholate), a polymer/amphiphile complex cannot form prior to the mixing step

Table 1 shows the average particle diameters measured by laser diffraction on three replicate suspension preparations An initial size determination was made, after which the sample was sonicated for 1 minute The size determination was then repeated. The large size reduction upon sonication of Method A was indicative of particle aggregation

Table 1

Method	Concentrate	Aqueous Diluent	Average particle diameter (microns)	After sonication (1 minute)
A	itraconazole (18%),N-methyl-2-pyrrolidinone (6 mL)	poloxamer 188 (2 3%),sodium deoxycholate (0 3%)tris buffer (5 mM, pH 8)water (qs to 94 mL)	18 7 10 7 12 1	2.36 2 46 1 93
B	itraconazole (18%)poloxamer 188 (37%)N-methyl-2-pyrrolidinone (6 mL)	sodium deoxycholate (0 3%)tris buffer (5 mM pH 8)water (qs to 94 mL)	0 194 0 178 0 181	0 198 0 179 0 177

A drug suspension resulting from application of the processes described in this invention may be administered directly as an injectable solution provided Water for Injection is used in formulation and an appropriate means for solution sterilization is applied Sterilization may be accomplished by separate sterilization of the drug concentrate (drug, solvent, and optional surfactant) and the diluent medium (water, and optional buffers and surfactants) prior to mixing to form the pre-suspension Sterilization methods would include pre-filtration first through a 3 0

micron filter followed by filtration through a 0 45-micron particle filter, followed by steam or heat sterilization or sterile filtration through two redundant 0 2-micron membrane filters

Optionally a solvent free suspension may be produced by solvent removal after precipitation This can be accomplished by centrifugation dialysis, diafiltration, force-field fractionation high pressure filtration or other separation techniques well known in the art Complete removal of N-methyl-2-pyrrolidinone was typically carried out by one to three successive centrifugation runs after each centrifugation (18,000 rpm for 30 minutes) the supernatant was decanted and discarded A fresh volume of the suspension vehicle without the organic solvent was added to the remaining solids and the mixture was dispersed by homogenization It will be recognized by others skilled in the art that other high-shear mixing techniques could be applied in this reconstitution step

Furthermore, any undesired excipients such as surfactants may be replaced by a more desirable excipient by use of the separation methods described in the above paragraph The solvent and first excipient may be discarded with the supernatant after centrifugation or filtration A fresh volume of the suspension vehicle without the solvent and without the first excipient may then be added. Alternatively, a new surfactant may be added For example, a suspension consisting of drug N methyl-2-pyrrolidinone (solvent) poloxamer 188 (first excipient), sodium deoxycholate, glycerol and water may be replaced with phospholipids (new surfactant) glycerol and water after centrifugation and removal of the supernatant.

I First Process Category

The methods of the first process category generally include the step of dissolving the organic compound in a water miscible first solvent followed by the step of mixing this solution with an aqueous solution to form a presuspension wherein the organic compound is in an amorphous form a semicrystalline form or in a supercooled liquid form as determined by x-ray diffraction studies DSC, light microscopy or other analytical techniques and has an average effective particle size within one of the effective particle size ranges set forth above The mixing step is followed by an energy-addition step and, in a preferred form of the invention an annealing step

II Second Process Category

The methods of the second processes category include essentially the same steps as in the steps of the first processes category but differ in the following respect. An x-ray diffraction DSC or other suitable analytical techniques of the presuspension shows the organic compound in a crystalline form and having an average effective particle size The organic compound after the energy-addition step has essentially the same average effective particle size as prior to the energy-

addition step but has less of a tendency to aggregate into larger particles when compared to that of the particles of the presuspension Without being bound to a theory it is believed the differences in the particle stability may be due to a reordering of the surfactant molecules at the solid liquid interface

III Third Process Category

The methods of the third category modify the first two steps of those of the first and second processes categories to ensure the organic compound in the presuspension is in a friable form having an average effective particle size (e g , such as slender needles and thin plates) Friable particles can be formed by selecting suitable solvents surfactants or combination of surfactants the temperature of the individual solutions, the rate of mixing and rate of precipitation and the like Friability may also be enhanced by the introduction of lattice defects (e g cleavage planes) during the steps of mixing the first solution with the aqueous solution This would arise by rapid crystallization such as that afforded in the precipitation step In the energy-addition step these friable crystals are converted to crystals that are kinetically stabilized and having an average effective particle size smaller than those of the presuspension Kinetically stabilized means particles have a reduced tendency to aggregate when compared to particles that are not kinetically stabilized In such instance the energy-addition step results in a breaking up of the friable particles By ensuring the particles of the presuspension are in a friable state, the organic compound can more easily and more quickly be prepared into a particle within the desired size ranges when compared to processing an organic compound where the steps have not been taken to render it in a friable form

Polymorph Control

The present invention further provides additional steps for controlling the crystal structure of the pharmaceutically-active compound to ultimately produce a suspension of the compound in the desired size range and a desired crystal structure What is meant by the term "crystal structure" is the arrangement of the atoms within the unit cell of the crystal Pharmaceutically-active compounds that can be crystallized into different crystal structures are said to be polymorphic. Identification of polymorphs is important step in drug formulation since different polymorphs of the same drug can show differences in solubility, therapeutic activity, bioavailability and suspension stability Accordingly, it is important to control the polymorphic form of the compound for ensuring product purity and batch-to-batch reproducibility

The steps to control the polymorphic form of the compound includes seeding the first solution, the second solvent or the presuspension to ensure the formation of the desired polymorph Seeding includes using a seed compound or adding energy In a preferred form of the invention the seed compound is the pharmaceutically-active compound in the desired polymorphic form

Alternatively the seed compound can also be an inert impurity or an organic compound with a structure similar to that of the desired polymorph such as a bile salt.

The seed compound can be precipitated from the first solution This method includes the steps of adding the pharmaceutically-active compound in sufficient quantity to exceed the solubility of the pharmaceutically active compound in the first solvent to create a supersaturated solution The supersaturated solution is treated to precipitate the pharmaceutically-active compound in the desired polymorphic form Treating the supersaturated solution includes aging the solution for a time period until the formation of a crystal or crystals is observed to create a seeding mixture It is also possible to add energy to the supersaturated solution to cause the pharmaceutically-active compound to precipitate out of the solution in the desired polymorph The energy can be added in a variety of ways including the energy addition steps described above Further energy can be added by heating or exposing the presuspension to electromagnetic energy, particle beam, or electron beam sources The electromagnetic energy includes using a laser beam, dynamic electromagnetic energy, or other radiation sources It is further contemplated utilizing ultrasound static electric field and a static magnetic field as the energy addition source

In a preferred form of the invention, the method for producing seed crystals from an aged supersaturated solution includes the steps of (i) adding a quantity of the pharmaceutically-active compound to the first organic solvent to create a supersaturated solution, (ii) aging the supersaturated solution to form detectable crystals to create a seeding mixture and (iii) mixing the seeding mixture with the second solvent to precipitate the pharmaceutically-active compound to create a presuspension The presuspension can then be further processed as described in detail above to provide an aqueous suspension of the pharmaceutically-active compound in the desired polymorph and in the desired size range

Seeding can also be accomplished by adding energy to the first solution, the second solvent or the presuspension provided that the exposed liquid or liquids contain the pharmaceutically active compound or a seed material The energy can be added in the same fashion as described above for the supersaturated solution

Accordingly the present invention provides a composition of matter of a pharmaceutically active compound in a desired polymorphic form essentially free of the unspecified polymorph or polymorphs One such example is set forth in example 16 below where seeding during microprecipitation provides a polymorph of itraconazole essentially free of the polymorph of the raw material It is contemplated the methods of this invention can apply used to selectively produce a desired polymorph for numerous pharmaceutically active compounds

Examples

Examples of Process Category 1

Example 1 Preparation of itraconazole suspension by use of Process Category 1 Method A with homogenization

To a 3-L flask add 1680 mL of Water for Injection Heat liquid to 60-65°C and then slowly add 44 grams of Pluronic F-68 (poloxamer 188) and 12 grams of sodium deoxycholate, stirring after each addition to dissolve the solids After addition of solids is complete stir for another 15 minutes at 60-65°C to ensure complete dissolution Prepare a 50 mM tris (tromethamine) buffer by dissolving 6 06 grams of tris in 800 ml of Water for Injection Titrate this solution to pH 8 0 with 0 1 M hydrochloric acid. Dilute the resulting solution to 1 liter with additional Water for Injection Add 200 mL of the tris buffer to the poloxamer/deoxycholate solution Stir thoroughly to mix solutions

In a 150-mL beaker add 20 grams of itraconazole and 120 mL of N-methyl-2-pyrrolidinone Heat mixture to 50-60°C, and stir to dissolve solids After total dissolution is visually apparent stir another 15 minutes to ensure complete dissolution Cool itraconazole-NMP solution to room temperature

Charge a syringe pump (two 60-mL glass syringes) with the 120-mL of itraconazole solution prepared previously Meanwhile pour all of the surfactant solution into a homogenizer hopper which has been cooled to 0-5°C (this may either by accomplished by use of a jacketed hopper through which refrigerant is circulated, or by surrounding the hopper with ice) Position a mechanical stirrer into the surfactant solution so that the blades are fully immersed Using the syringe pump slowly (1-3 mL/min) add all of the itraconazole solution to the stirred, cooled surfactant solution A stirring rate of at least 700 rpm is recommended An aliquot of the resulting suspension (Suspension A) is analyzed by light microscopy (Hoffman Modulation Contrast) and by laser diffraction (Horiba) Suspension A is observed by light microscopy to consist of roughly spherical amorphous particles (under 1 micron), either bound to each other in aggregates or freely moving by Brownian motion See Figure 3 Dynamic light scattering measurements typically afford a bimodal distribution pattern signifying the presence of aggregates (10-100 microns in size) and the presence of single amorphous particles ranging 200-700 nm in median particle diameter

The suspension is immediately homogenized (at 10,000 to 30,000 psi) for 10-30 minutes At the end of homogenization, the temperature of the suspension in the hopper does not exceed 75°C The homogenized suspension is collected in 500-mL bottles, which are cooled immediately in the refrigerator (2-8°C) This suspension (Suspension B) is analyzed by light microscopy and is found to consist of small elongated plates with a length of 0 5 to 2 microns and a width in the 0 2-1 micron range See Figure 4 Dynamic light scattering measurements typically indicate a median diameter

of 200-700 nm.

Figure 3 Amorphous particles prior to homogenization (Example 1)

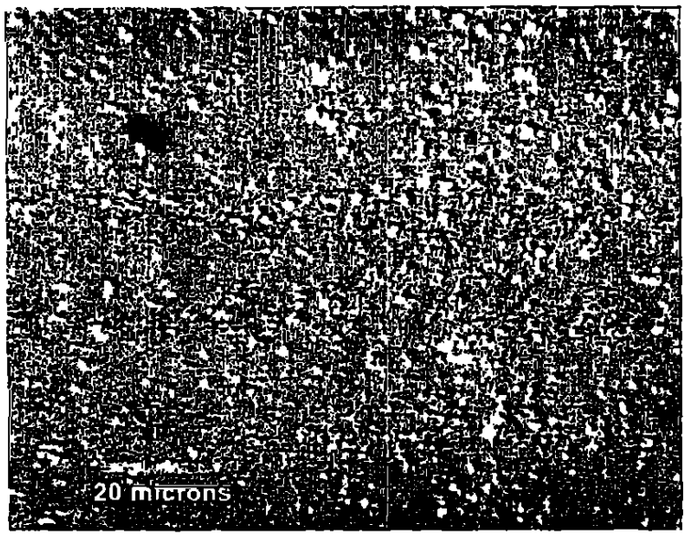


Figure 4 Particles after annealing by homogenization

Stability of Suspension A ("Pre-suspension") (Example 1)

During microscopic examination of the aliquot of Suspension A crystallization of the amorphous solid was directly observed Suspension A was stored at 2 8 °C for 12 hours and examined by light microscopy Gross visual inspection of the sample revealed severe flocculation,

with some of the contents settling to the bottom of the container Microscopic examination indicated the presence of large, elongated plate like crystals over 10 microns in length

Stability of Suspension B

As opposed to the instability of Suspension A, Suspension B was stable at 2-8°C for the duration of the preliminary stability study (1 month) Microscopy on the aged sample clearly demonstrated that no significant change in the morphology or size of the particles had occurred This was confirmed by light scattering measurement.

Example 2 Preparation of itraconazole suspension by use of Process Category 1, Method A with ultrasonication

To a 500-mL stainless steel vessel add 252 mL of Water for Injection Heat liquid to 60 65°C, and then slowly add 6 6 grams of Pluronic F-68 (poloxamer 188), and 0 9 grams of sodium deoxycholate stirring after each addition to dissolve the solids After addition of solids is complete stir for another 15 minutes at 60-65°C to ensure complete dissolution Prepare a 50 mM tris (tromethamine) buffer by dissolving 6 06 grams of tris in 800 mL of Water for Injection Titrate this solution to pH 8 0 with 0 1 M hydrochloric acid Dilute the resulting solution to 1 liter with additional Water for Injection Add 30 mL of the tris buffer to the poloxamer/deoxycholate solution Stir thoroughly to mix solutions

In a 30-mL container add 3 grams of itraconazole and 18 mL of N-methyl-2 pyrrolidinone Heat mixture to 50-60°C, and stir to dissolve solids After total dissolution is visually apparent, stir another 15 minutes to ensure complete dissolution Cool itraconazole-NMP solution to room temperature

Charge a syringe pump with 18-mL of itraconazole solution prepared in a previous step Position a mechanical stirrer into the surfactant solution so that the blades are fully immersed Cool the container to 0-5°C by immersion in an ice bath Using the syringe pump, slowly (1-3 mL/min) add all of the itraconazole solution to the stirred, cooled surfactant solution A stirring rate of at least 700 rpm is recommended. Immerse an ultrasonicator horn in the resulting suspension so that the probe is approximately 1 cm above the bottom of the stainless steel vessel Sonicate (10,000 to 25 000 Hz, at least 400W) for 15 to 20 minute in 5-minute intervals After the first 5-minute sonication remove the ice bath and proceed with further sonication At the end of ultrasonication, the temperature of the suspension in the vessel does not exceed 75°C

The suspension is collected in a 500-mL Type I glass bottle, which is cooled immediately in the refrigerator (2-8°C) Characteristics of particle morphology of the suspension before and after sonication were very similar to that seen in Method A before and after homogenization (see Example 1)

Example 3 Preparation of itraconazole suspension by use of Process Category 1, Method B with homogenization

Prepare a 50 mM tris (tromethamine) buffer by dissolving 6 06 grams of tris in 800 mL of Water for Injection Titrate this solution to pH 8 0 with 0 1 M hydrochloric acid Dilute the resulting solution to 1 liter with additional Water for Injection. To a 3 L flask add 1680 mL of Water for Injection Add 200 mL of the tris buffer to the 1680 mL of water Stir thoroughly to mix solutions

In a 150-mL beaker add 44 grams of Pluronic F-68 (poloxamer 188) and 12 grams of sodium deoxycholate to 120 mL of N-methyl-2-pyrrolidinone. Heat the mixture to 50-60°C, and stir to dissolve solids After total dissolution is visually apparent stir another 15 minutes to ensure complete dissolution To this solution add 20 grams of itraconazole, and stir until totally dissolved Cool the itraconazole-surfactant-NMP solution to room temperature.

Charge a syringe pump (two 60-mL glass syringes) with the 120-mL of the concentrated itraconazole solution prepared previously Meanwhile pour the diluted tris buffer solution prepared above into a homogenizer hopper which has been cooled to 0 5°C (this may either by accomplished by use of a jacketed hopper through which refrigerant is circulated, or by surrounding the hopper with ice) Position a mechanical stirrer into the buffer solution so that the blades are fully immersed. Using the syringe pump, slowly (1 3 mL/min) add all of the itraconazole-surfactant concentrate to the stirred, cooled buffer solution. A stirring rate of at least 700 rpm is recommended. The resulting cooled suspension is immediately homogenized (at 10,000 to 30 000 psi) for 10-30 minutes At the end of homogenization the temperature of the suspension in the hopper does not exceed 75°C

The homogenized suspension is collected in 500-mL bottles which are cooled immediately in the refrigerator (2-8°C) Characteristics of particle morphology of the suspension before and after homogenization were very similar to that seen in Example 1, except that in process category 1 B, the pre-homogenized material tended to form fewer and smaller aggregates which resulted in a much smaller overall particle size as measured by laser diffraction. After homogenization, dynamic light scattering results were typically identical to those presented in Example 1

Example 4 Preparation of itraconazole suspension by use of Process Category 1, Method B with ultrasonication

To a 500-mL flask add 252 ml of Water for Injection Prepare a 50 mM tris (tromethamine) buffer by dissolving 6 06 grams of tris in 800 mL of Water for Injection Titrate this solution to pH 8 0 with 0 1 M hydrochloric acid Dilute the resulting solution to 1 liter with additional Water for Injection Add 30 mL of the tris buffer to the water Stir thoroughly to mix solutions

In a 30-mL beaker add 6 6 grams of Pluronic F-68 (poloxamer 188) and 0 9 grams of sodium deoxycholate to 18 mL of N-methyl-2 pyrrolidinone Heat the mixture to 50-60°C, and stir to dissolve solids After total dissolution is visually apparent, stir another 15 minutes to ensure complete dissolution To this solution add 3 0 grams of itraconazole and stir until totally dissolved. Cool the itraconazole-surfactant-NMP solution to room temperature

Charge a syringe pump (one 30-mL glass syringe with the 18-mL of the concentrated itraconazole solution prepared previously Position a mechanical stirrer into the buffer solution so that the blades are fully immersed Cool the container to 0-5°C by immersion in an ice bath Using the syringe pump, slowly (1-3 mL/min) add all of the itraconazole-surfactant concentrate to the stirred, cooled buffer solution A stirring rate of at least 700 rpm is recommended The resulting cooled suspension is immediately sonicated (10,000 to 25,000 Hz, at least 400 W) for 15-20 minutes, in 5-minute intervals After the first 5-minute sonication, remove the ice bath and proceed with further sonication At the end of ultrasonication, the temperature of the suspension in the hopper does not exceed 75°C

The resultant suspension is collected in a 500-mL bottle, which is cooled immediately in the refrigerator (2-8°C) Characteristics of particle morphology of the suspension before and after sonication were very similar to that seen in Example 1, except that in Process Category 1, Method B, the pre-sonicated material tended to form fewer and smaller aggregates which resulted in a much smaller overall particle size as measured by laser diffraction After ultrasonication, dynamic light scattering results were typically identical to those presented in Example 1

B Examples of Process Category 2

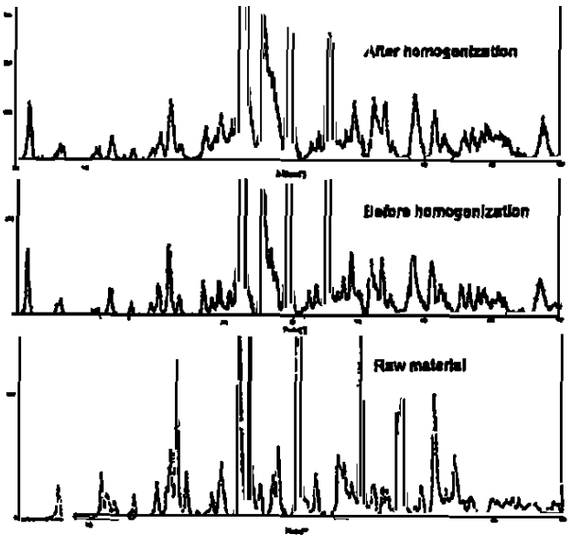
Example 5 Preparation of itraconazole suspension (1%) with 0 75% Solutol® HR (PEG-660 12-hydroxystearate) Process Category 2, Method B

Solutol (2 25 g) and itraconazole (3 0 g) were weighed into a beaker and 36 mL of filtered N methyl-2-pyrrolidinone (NMP) was added This mixture was stirred under low heat (up to 40°C) for approximately 15 minutes until the solution ingredients were dissolved The solution was cooled to room temperature and was filtered through a 0 2-micron filter under vacuum. Two 60-mL syringes were filled with the filtered drug concentrate and were placed in a syringe pump The pump was set to deliver approximately 1 mL/min of concentrate to a rapidly stirred (400 rpm) aqueous buffer solution The buffer solution consisted of 22 g/L of glycerol in 5 mM tris buffer Throughout concentrate addition the buffer solution was kept in an ice bath at 2-3°C At the end of the precipitation, after complete addition of concentrate to the buffer solution, about 100 mL of the suspension was centrifuged for 1 hour, the supernatant was discarded The precipitate was resuspended in a 20% NMP solution in water, and again centrifuged for 1 hour The material was

dried overnight in a vacuum oven at 25°C The dried material was transferred to a vial and analyzed by X ray diffractometry using chromium radiation (see Figure 5)

Another 100 mL-aliquot of the microprecipitated suspension was sonicated for 30 minutes at 20 000 Hz 80% full amplitute (full amplitude = 600 W) The sonicated sample was homogenized in 3 equal aliquots each for 45 minutes (Avestin C5 2 5°C 15,000-20,000 psi) The combined fractions were centrifuged for abou 3 hours the supernatant removed and the precipitate resuspended in 20% NMP The resuspended mixture was centrifuged again (15,000 rpm at 5°C) The supernatant was decanted off and the precipitate was vacuum dried overnight at 25°C The precipitate was submitted for analysis by X-ray diffractometry (see Figure 5) As seen in Figure 5 the X-ray diffraction patterns of processed samples before and after homogenization, are essentially identical, yet show a significantly different pattern as compared with the starting raw material The unhomogenized suspension is unstabl and agglomerates upon storage at room temperature The stabilization that occurs as a result of homogenization is believed to arise from rearrangement of surfactant on the surface of the particle This rearrangement should result in a lower propensity for particle aggregation

Figure 5 X-Ray diffractogram of microprecipitated itraconazole with polyethylene glycol-660 12-hydroxystearate before and after homogenization (Example 5)



C Examples of Process Category

Example 6 Preparation of carbamazepine suspension by use of Process Category 3, Method A with homogenization

2 08 g of carbamazepine was dissolved into 10 mL of NMP 1 0 mL of this concentrate was subsequently dripped at 0 1 mL/min into 20 mL of a stirred solution of 1 2% lecithin and 2 25% glycerin The temperature of the lecithin system was held at 2-5°C during the entire addition The predispersion was next homogenized cold (5-15°C) for 35 minutes at 15,000 psi The pressure was increased to 23,000 psi and the homogenization was continued for another 20 minutes The particles produced by the process had a mean diameter of 0 881 µm with 99% of the particles being less than 2 44 um

Example 7 Preparation of 1% carbamazepine suspension with 0 125% Solutol® by use of Process Category 3, Method B with homogenization

A drug concentrate of 20% carbamazepine and 5% glycodeoxycholic acid (Sigma Chemical Co) in N-methyl-2-pyrrolidinone was prepared The microprecipitation step involved adding the drug concentrate to the receiving solution (distilled water) at a rate of 0 1 mL/min The receiving solution was stirred and maintained at approximately 5° C during precipitation After precipitation, the final ingredient concentrations were 1% carbamazepine and 0 125% Solutol® The drug crystals were examined under a light microscope using positive phase contrast (400X) The precipitate consisted of fine needles approximately 2 microns in diameter and ranging from 50 – 150 microns in length

Homogenization (Avestin C 50 piston-gap homogenizer) at approximately 20,000 psi for approximately 15 minutes results in small particles less than 1 micron in size and largely unaggregated. Laser diffraction analysis (Horiba) of the homogenized material showed that the particles had a mean size of 0 4 micron with 99% of the particles less than 0 8 micron Low energy sonication, suitable for breaking agglomerated particles but not with sufficient energy to cause a comminution of individual particles, of the sample before Horiba analysis had no effect on the results (numbers were the same with and without sonication) This result was consistent with the absence of particle agglomeration

Samples prepared by the above process were centrifuged and the supernatant solutions replaced with a replacement solution consisting of 0 125% Solutol® After centrifugation and supernatant replacement, the suspension ingredient concentrations were 1% carbamazepine and 0 125% Solutol® The samples were re-homogenized by piston-gap homogenizer and stored at 5° C After 4 weeks storage, the suspension had a mean particle size of 0 751 with 99% less than 1 729 Numbers reported are from Horiba analysis on unsonicated samples

Example 8 Preparation of 1% carbamazepine suspension with 0 06% sodium glycodeoxycholate and 0 06% poloxamer 188 by use of Process Category 3, Method B with homogenization

A drug concentrate comprising 20% carbamazepine and 5% glycodeoxycholate in N-methyl-2-pyrrolidinone was prepared The microprecipitation step involved adding the drug concentrate to the receiving solution (distilled water) at a rate of 0 1 mL/min Thus, this and the following examples demonstrate that adding a surfactant or other excipient to the aqueous precipitating solution in Methods A and B above is optional The receiving solution was stirred and maintained at approximately 5° C during precipitation After precipitation the final ingredient concentrations were 1% carbamazepine and 0 125% Solutol® The drug crystals were examined under a light microscope using positive phase contrast (400X) The precipitate consisted of fine needles approximately 2 microns in diameter and ranging from 50 – 150 microns in length Comparison of the precipitate with the raw material before precipitation reveals that the precipitation step in the presence of surface modifier (glycodeoxycholic acid) results in very slender crystals that are much thinner than the starting raw material (see Figure 6)

Homogenization (Avestin C-50 piston-gap homogenizer) at approximately 20 000 psi for approximately 15 minutes results in small particles less than 1 micron in size and largely unaggregated See Figure 7 Laser diffraction analysis (Horiba) of the homogenized material showed that the particles had a mean size of 0 4 micron with 99% of the particles less than 0 8 micron Sonication of the sample before Horiba analysis had no effect on the results (numbers were the same with and without sonication) This result was consistent with the absence of particle agglomeration

Samples prepared by the above process were centrifuged and the supernatant solutions replaced with a replacement solution consisting of 0 06% glycodeoxycholic acid (Sigma Chemical Co) and 0 06% Poloxamer 188 The samples were re-homogenized by piston-gap homogenizer and stored at 5° C After 2 weeks storage, the suspension had a mean particle size of 0 531 micron with 99% less than 1 14 micron Numbers reported are from Horiba analysis on unsonicated samples

- - -

Figure 6 Carbamazepine crystals before homogenization (Example 6)

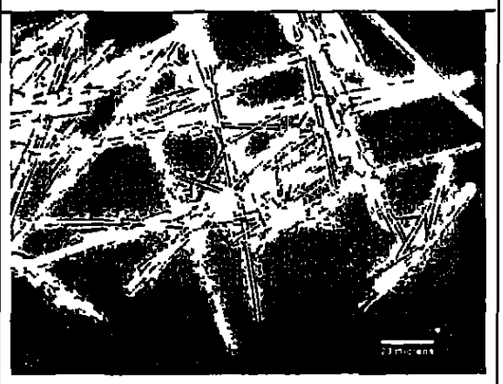
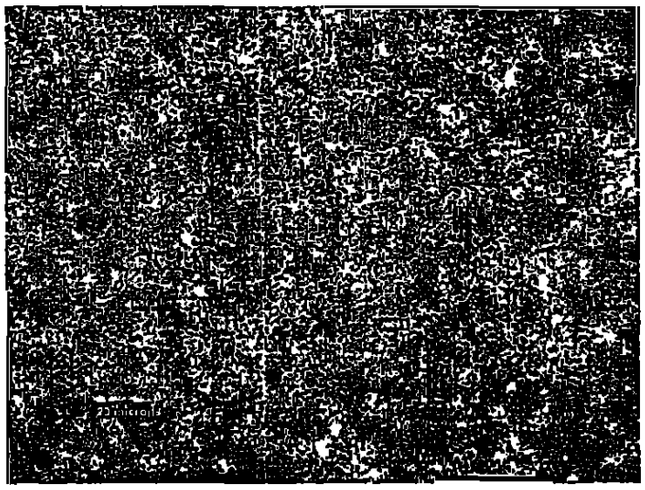
Raw material, before precipitation	After precipitation
	

Figure 7 Carbamazepine microparticulate after homogenization (Avestin C-50) (Example 6)



Mathematical Analysis (Example 8) of force required to break precipitated particles as compared to force required to break particles of the starting raw material (carbamazepine)

The width of the largest crystals seen in the carbamazepine raw material (Figure 6 picture on left) are roughly 10-fold greater than the width of crystals in the microprecipitated material (Figure 6, picture on right) On the assumption that the ratio of crystal thickness (1 10) is proportional to the ratio of crystal width (1 10), then the moment of force required to cleave the larger crystal in the raw material should be approximately 1,000-times greater than the force needed to break the microprecipitated material, since

$$e_L = 6PL/(Ewx^2) \qquad \text{Eq 1}$$

where,

e_L = longitudinal strain required to break the crystal ("yield value")

P = load on beam

L = distance from load to fulcrum

E = elasticity modulus

w = width of crystal

x = thickness of crystal

Let us assume that L and E are the same for the raw material and the precipitated material Additionally, let us assume that $w/w_C = x/x_0 = 10$ Then,

$(e_L)_0 = 6P_0L/(Ew_0x_0^2)$ when the 0 subscripts refer to raw material

$e_L = 6PL/(Ewx^2)$ for the microprecipitate

Equating $(e_L)_0$ and e_L

$6PL/(Ewx^2) = 6P_0L/(Ew_0x_0^2)$

After simplification

$P = P_0 (w/w_0) (x/x_0)^2 = P_0 (0\ 1) (0\ 1)^2 = 0\ 001\ P_0$

Thus the yield force, P required to break the microprecipitated solid is one thousandth the required force necessary to break the starting crystalline solid If, because of rapid precipitation lattice defects or amorphic properties are introduced, then the modulus (E) should decrease, making the microprecipitate even easier to cleave

Example 9 Preparation of 1 6% (w/v) prednisolone suspension with 0 05% sodium deoxycholate and 3% N-methyl-2 pyrrolidinone Process Category 3, Method B

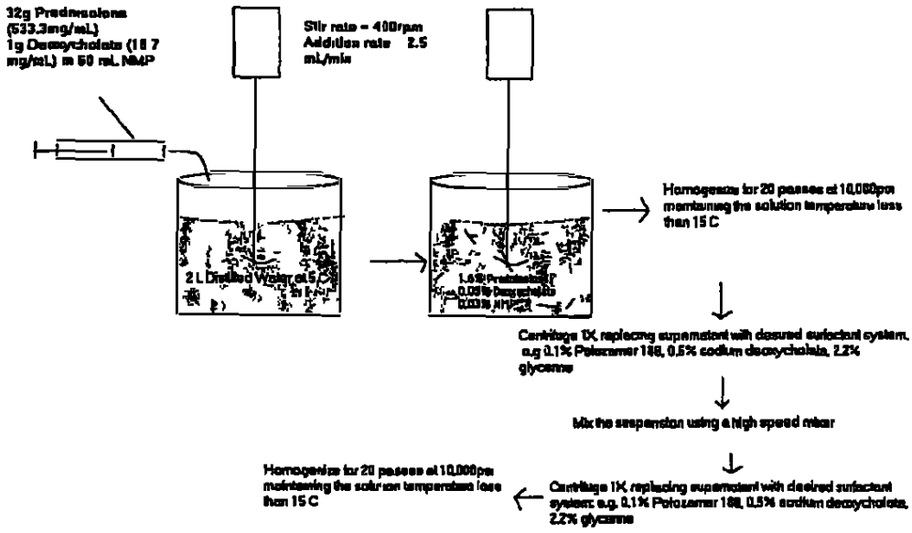
A schematic of the overall manufacturing process is presented in Figure 8 A concentrated solution of prednisolone and sodium deoxycholate was prepared Prednisolone (32g) and sodium deoxycholate (1g) were added to a sufficient volume of 1-methyl 2-pyrrolidinone (NMP) to produce a final volume of 60 mL. The resulting prednisolone concentration was approximately 533 3 mg/mL and the sodium deoxycholate concentration was approximately 16 67 mg/mL 60mL of NMP concentrate was added to 2 L of water cooled to 5°C at an addition rate of 2 5 mL/min while stirring at approximately 400 rpm The resulting suspension contained slender needle shaped crystals less than 2 µm in width (Figure 9) The concentration contained in the precipitated suspension was 1 6% (w/v) prednisolone 0 05% sodium deoxycholate, and 3% NMP

The precipitated suspension was pH adjusted to 7.5-8 5 using sodium hydroxide and hydrochloric acid then homogenized (Avestin C-50 piston-gap homogenizer) for 10 passes at 10,000 psi The NMP was removed by performing 2 successive centrifugation steps replacing the supernatant each time with a fresh surfactant solution, which contained the desired concentrations of

surfactants needed to stabilize the suspension (see Table 2) The suspension was homogenized for another 10 passes at 10 000 psi The final suspension contained particles with a mean particle size of less than 1 µm, and 99% of particles less than 2 µm Figure 10 is a photomicrograph of the final prednisolone suspension after homogenization

A variety of different surfactants at varying concentrations were used in the centrifugation/surfactant replacement step (see Table 2) Table 2 lists combinations of surfactants that were stable with respect to particle size (mean < 1 µm, 99%< 2 µm), pH (6-8) drug concentration (less than 2% loss) and re-suspendability (resuspended in 60 seconds or less)

Figure 8 Diagram of Microprecipitation Process for Prednisolone (Examples 9-12)



Notably this process allows for adding the active compound to an aqueous diluent without the presence of a surfactant or other additive. This is a modification of process Method B in Figure 2

Figure 9 Photomicrograph of prednisolone suspension before homogenization (Hoffman Modulation Contrast, 1250X magnification)

Figure 10 Photomicrograph of prednisolone suspension after homogenization (Hoffman Modulation Contrast, 1250X magnification)

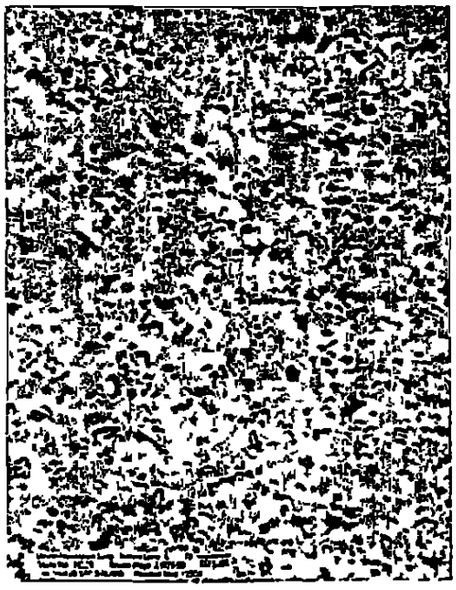


Table 2 List of stable prednisolone suspensions prepared by microprecipitation process of Figure 8 (Example 9)

			2 Weeks		2 Months						
	Initial		40°C		5°C		25°C		40°C		
Formulation	Mean	>99%	Mean	>99%	Mean	>99%	Mean	>99%	Mean	>99%	% Loss*
1 6% prednisolone 0 6% phospholipids, 0 5% sodium deoxycholate, 5 mM TRIS 2.2% glycerol **	0 79	1 65	0 84	1 79	0.83	1 86	0 82	1 78	0 82	1 93	<2%
1 6% prednisolone 0 6% Solutol® 0.5% sodium deoxycholate, 2.2% glycerol	0 77	1.52	0 79	1 67	0 805	1 763	0 796	1 693	0 81	1 633	<2%
1 6% prednisolone 0 1% poloxamer 188 0 5% sodium deoxycholate, 2.2% glycerol	0 64	1 16	0 82	1 78	0 696	1 385	0 758	1 698	0 719	1 473	<2%
1 6% prednisolone 5% phospholipids 5 mM TRIS 2 2% glycerol	0 824	1 77	0 87	1 93	0 88	1 95	0 869	1 778	0 909	1 993	<2%

* Difference in itraconazole concentration between samples stored for 2 months at 5 and 25°C

** Stable through at least 6 months

Particle sizes (by laser light scattering), in microns

5°C 0 80 (mean), 1 7 (99%)

25°C 0 90 (mean) 2 51 (99%)

40°C 0 99 (mean), 2 03 (99%)

Difference in itraconazole concentration between samples stored at 5 and 25°C <2%

Example 10 Preparation of prednisolone suspension by use of Process Category 3, Method A with homogenization

32 g of prednisolone was dissolved into 40 mL of NMP Gentle heating at 40-50°C was required to effect dissolution The drug NMP concentrate was subsequently dripped at 2 5 mL/min into 2 liters of a stirred solution that consisted of 0 1 2% lecithin and 2 2% glycerin No other surface modifiers were added. The surfactant system was buffered at pH = 8 0 with 5 mM tris buffer and the temperature was held at 0° to 5°C during the entire precipitation process The post-precipitated dispersion was next homogenized cold (5-15 °C) for 20 passes at 10,000 psi Following homogenization, the NMP was removed by centrifuging the suspension, removing the supernatant, and replacing the supernatant with fresh surfactant solution This post-centrifuged suspension was then rehomogenized cold (5-15 °C) for another 20 passes at 10,000 psi The particles produced by this process had a mean diameter of 0 927 um with 99% of the particles being less than 2 36 um.

Example 11 Preparation of nabumetone suspension by use of Process Category 3 Method B with homogenization

Surfactant (2 2 g of poloxamer 188) was dissolved in 6 mL of N-methyl-2-pyrrolidinone This solution was stirred at 45°C for 15 minutes after which 1 0 g of nabumetone was added The drug dissolved rapidly Diluent was prepared which consisted of 5 mM tris buffer with 2 2% glycerol and adjusted to pH 8 A 100-mL portion of diluent was cooled in an ice bath The drug concentrate was slowly added (approximately 0 8 mL/min) to the diluent with vigorous stirring This crude suspension was homogenized at 15 000 psi for 30 minutes and then at 20,000 psi for 30 minutes (temperature = 5°C) The final nanosuspension was found to be 930 nm in effective mean diameter (analyzed by laser diffraction) 99% of the particles were less than approximately 2 6 microns

Example 12 Preparation of nabumetone suspension by use of Process Category 3 Method B with homogenization and the use of Solutol® HS 15 as the surfactant. Replacement of supernatant liquid with a phospholipid medium.

Nabumetone (0 987 grams) was dissolved in 8 mL of N-methyl-2-pyrrolidinone To this solution was added 2 2 grams of Solutol® HS 15 This mixture was stirred until complete dissolution of the surfactant in the drug concentrate Diluent was prepared which consisted of 5 mM tris buffer with 2 2% glycerol, and which was adjusted to pH 8 The diluent was cooled in an ice bath and the drug concentrate was slowly added (approximately 0.5 mL/min) to the diluent with vigorous stirring This crude suspension was homogenized for 20 minutes at 15 000 psi, and for 30 minutes at 20 000 psi

The suspension was centrifuged at 15 000 rpm for 15 minutes and the supernatant was removed and discarded. The remaining solid pellet was resuspended in a diluent consisting of 1 2% phospholipids This medium was equal in volume to the amount of supernatant removed in the previous step The resulting suspension was then homogenized at approximately 21,000 psi for 30 minutes The final suspension was analyzed by laser diffraction and was found to contain particles with a mean diameter of 542 nm, and a 99% cumulative particle distribution sized less than 1 micron

Example 13 Preparation of 1% itraconazole suspension with poloxamer with particles of a mean diameter of approximately 220 nm

Itraconazole concentrate was prepared by dissolving 10 02 grams of itraconazole in 60 mL of N methyl-2-pyrrolidinone. Heating to 70°C was required to dissolve the drug The solution was then cooled to room temperature A portion of 50 mM tris(hydroxymethyl)aminomethane buffer

(tris buffer) was prepared and was pH adjusted to 8 0 with 5M hydrochloric acid An aqueous surfactant solution was prepared by combining 22 g/L poloxamer 407 3 0 g/L egg phosphatides 22g/L glycerol, and 3 0 g/L sodium cholate dihydrate 900 mL of the surfactant solution was mixed with 100 mL of the tris buffer to provide 1000 mL of aqueous diluent

The aqueous diluent was added to the hopper of the homogenizer (APV Gaulin Model 15MR-8TA) which was cooled by using an ice jacket The solution was rapidly stirred (4700 rpm) and the temperature was monitored The itraconazole concentrate was slowly added, by use of a syringe pump, at a rate of approximately 2 mL/min Addition was complete after approximately 30 minute The resulting suspension was stirred for another 30 minutes while the hopper was still being cooled in an ice jacket, and an aliquot was removed for analysis by light microscopy any dynamic light scatting The remaining suspension was subsequently homogenized for 15 minutes at 10 000 psi By the end of the homogenization the temperature had risen to 74°C The homogenized suspension was collected in a 1-L Type I glass bottle and sealed with a rubber closure The bottle containing suspension was stored in a refrigerator at 5°C

A sample of the suspension before homogenization showed the sample to consist of both free particles, clumps of particles, and multilamellar lipid bodies The free particles could not be clearly visualized due to Brownian motion, however many of the aggregates appeared to consist of amorphous, non-crystalline material

The homogenized sample contained free submicron particles having excellent size homogeneity without visible lipid vesicles Dynamic light scattering showed a monodisperse logarithmic size distribution with a median diameter of approximately 220 nm. The upper 99% cumulative size cutoff was approximately 500 nm Figure 11 shows a comparison of the size distribution of the prepared nanosuspension with that of a typical parenteral fat emulsion product (10% Intralipid®, Pharmacia)

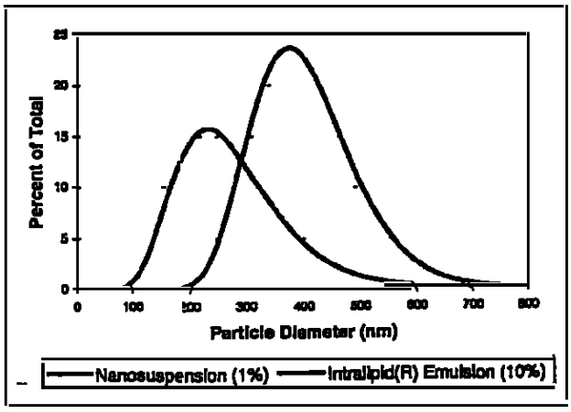


Figure 11 Comparison of size distributions of nanosuspensions (this invention) and commercial fat emulsion (Example 13)

Example 14 Preparation of 1% itraconazole nanosuspension with hydroxyethylstarch

Preparation of Solution A Hydroxyethylstarch (1 g, Ajinomoto) was dissolved in 3 mL of N-methyl-2-pyrrolidinone (NMP) This solution was heated in a water bath to 70-80°C for 1 hour In another container was added 1 g of itraconazole (Wyckoff) Three mL of NMP were added and the mixture heated to 70-80°C to effect dissolution (approximately 30 minutes) Phospholipid (Lipoid S-100) was added to this hot solution Heating was continued at 70-90°C for 30 minutes until all of the phospholipid was dissolved The hydroxyethylstarch solution was combined with the itraconazole/ phospholipid solution This mixture was heated for another 30 minutes at 80-95°C to dissolve the mixture

Addition of Solution A to Tris Buffer Ninety-four (94) mL of 50 mM tris(hydroxymethyl)aminomethane buffer was cooled in an ice bath As the tris solution was being rapidly stirred, the hot Solution A (see above) was slowly added dropwise (less than 2 cc/minute)

After complete addition the resulting suspension was sonicated (Cole-Parmer Ultrasonic Processor - 20 000 Hz, 80% amplitude setting) while still being cooled in the ice bath A one-inch solid probe was utilized Sonication was continued for 5 minutes The ice bath was removed, the probe was removed and retuned, and the probe was again immersed in the suspension The suspension was sonicated again for another 5 minutes without the ice bath The sonicator probe was once again removed and retuned, and after immersion of the probe the sample was sonicated for another 5 minutes At this point, the temperature of the suspension had risen to 82°C The suspension was quickly cooled again in an ice bath and when it was found to be below room temperature it was poured into a Type I glass bottle and sealed Microscopic visualization of the particles indicated individual particle sizes on the order of one micron or less

After one year of storage at room temperature, the suspension was reevaluated for particle size and found to have a mean diameter of approximately 300 nm.

Example 15 Prophetic example of Method A using HES

The present invention contemplates preparing a 1% itraconazole nanosuspension with hydroxyethylstarch utilizing Method A by following the steps of Example 14 with the exception the HES would be added to the tris buffer solution instead of to the NMP solution The aqueous solution may have to be heated to dissolve the HES

Example 16 Seeding during Homogenization to Convert a Mixture of Polymorphs to the More

#6 239

Stable Polymorph

Sample preparation An itraconazole nanosuspension was prepared by a microprecipitation-homogenization method as follows Itraconazole (3g) and Solutol HR (2 25g) were dissolved in 36mL of N-methyl-2-pyrrolidinone (NMP) with low heat and stirring to form a drug concentrate solution The solution was cooled to room temperature and filtered through a 0 2 μm nylon filter under vacuum to remove undissolved drug or particulate matter The solution was viewed under polarized light to ensure that no crystalline material was present after filtering. The drug concentrate solution was then added at 1 0 mL/minute to approximately 264 mL of an aqueous buffer solution (22 g/L glycerol in 5 mM tris buffer) The aqueous solution was kept at 2-3°C and was continuously stirred at approximately 400 rpm during the drug concentrate addition Approximately 100 mL of the resulting suspension was centrifuged and the solids re-suspended in a pre-filtered solution of 20% NMP in water This suspension was re-centrifuged and the solids were transferred to a vacuum oven for overnight drying at 25°C The resulting solid sample was labeled SMP 2 PRE

Sample characterization The sample SMP 2 PRE and a sample of the raw material itraconazole were analyzed using powder x-ray diffractometry The measurements were performed using a Rigaku MiniFlex+ instrument with copper radiation, a step size of 0 02° 2θ and scan speed of 0 25° 2θ/minute The resulting powder diffraction patterns are shown in Figure 12 The patterns show that SMP-2-PRE is significantly different from the raw material suggesting the presence of a different polymorph or a pseudopolymorph

Differential scanning calorimetry (DSC) traces for the samples are shown in Figures 13a and 13b Both samples were heated at 2°/min to 180°C in hermetically sealed aluminum pans

The trace for the raw material itraconazole (Figure 13a) shows a sharp endotherm at approximately 165°C

The trace for SMP 2 PRE (Figure 13b) exhibits two endotherms at approximately 159°C and 153°C This result, in combination with the powder x-ray diffraction patterns, suggests that SMP 2 PRE consists of a mixture of polymorphs and that the predominant form is a polymorph that is less stable than polymorph present in the raw material

Further evidence for this conclusion is provided by the DSC trace in Figure 14, which shows that upon heating SMP 2 PRE through the first transition, then cooling and reheating the less stable polymorph melts and recrystallizes to form the more stable polymorph

Seeding A suspension was prepared by combining 0 2g of the solid SMP 2 PRE and 0 2g of raw material itraconazole with distilled water to a final volume of 20 mL (seeded sample) The suspension was stirred until all the solids were wetted A second suspension was prepared in the same manner but without adding the raw material itraconazole (unseeded sample) Both suspensions were homogenized at approximately 18 000 psi for 30 minutes Final temperature of the

suspensions after homogenization was approximately 30°C The suspensions were then centrifuged and the solids dried for approximately 16 hours at 30°C

Figure 15 shows the DSC traces of the seeded and unseeded samples The heating rate for both samples was 2°/min to 180°C in hermetically sealed aluminum pans The trace for the unseeded sample shows two endotherms, indicating that a mixture of polymorphs is still present after homogenization The trace for the seeded sample shows that seeding and homogenization causes the conversion of the solids to the stable polymorph Therefore, seeding appears to influence the kinetics of the transition from the less stable to the more stable polymorphic form

Example 17 Seeding during Precipitation to Preferentially Form a Stable Polymorph

Sample preparation An itraconazole NMP drug concentrate was prepared by dissolving 1 67g of itraconazole in 10mL of NMP with stirring and gentle heating The solution was filtered twice using 0 2 μm syringe filters Itraconazole nanosuspensions were then prepared by adding 1 2 mL of the drug concentrate to 20 mL or an aqueous receiving solution at approx 3°C and stirring at approx 500 rpm. A seeded nanosuspension was prepared by using a mixture of approx 0 02g of raw material itraconazole in distilled water as the receiving solution An unseeded nanosuspension was prepared by using distilled water only as the receiving solution. Both suspensions were centrifuged, the supernatants decanted, and the solids dried in a vacuum oven at 30°C for approximately 16 hours

Sample characterization Figure 16 shows a comparison of the DSC traces for the solids from the seeded and unseeded suspensions The samples were heated at 2°/min to 180°C in hermetically sealed aluminum pans The dashed line represents the unseeded sample, which shows two endotherms indicating the presence of a polymorphic mixture

The solid line represents the seeded sample, which shows only one endotherm near the expected melting temperature of the raw material, indicating that the seed material induced the exclusive formation of the more stable polymorph

Example 18 Polymorph control by seeding the drug concentrate

Sample preparation The solubility of itraconazole in NMP at room temperature (approximately 22 °C) was experimentally determined to be 0 16 g/mL A 0 20 g/mL drug concentrate solution was prepared by dissolving 2 0 g of itraconazole and 0 2 g Poloxamer 188 in 10 mL NMP with heat and stirring This solution was then allowed to cool to room temperature to yield a supersaturated solution A microprecipitation experiment was immediately performed in which 1 5 mL of the drug concentrate was added to 30 mL of an aqueous solution containing 0 1% deoxycholate 2 2% glycerol The aqueous solution was maintained at ~2 °C and a stir rate of 350

rpm during the addition step The resulting presuspension was homogenized at ~13,000 psi for approx 10 minutes at 50 °C The suspension was then centrifuged, the supernatant decanted and the solid crystals dried in a vacuum oven at 30 °C for 135 hours

The supersaturated drug concentrate was subsequently aged by storing at room temperature in order to induce crystallization After 12 days, the drug concentrate was hazy, indicating that crystal formation had occurred An itraconazole suspension was prepared from the drug concentrate in the same manner as in the first experiment, by adding 1 5 mL to 30 mL of an aqueous solution containing 0 1% deoxycholate, 2 2% glycerol The aqueous solution was maintained at ~5 °C and a stir rate of 350 rpm during the addition step The resulting presuspension was homogenized at ~13 000 psi for approx 10 minutes at 50 °C The suspension was then centrifuged the supernatant decanted, and the solid crystals dried in a vacuum oven at 30 °C for 135 hours

Sample characterization X-ray powder diffraction analysis was used to determine the morphology of the dried crystals The resulting patterns are shown in Figure 17 The crystals from the first experiment (using fresh drug concentrate) were determined to consist of the more stable polymorph In contrast, the crystals from the second experiment (aged drug concentrate) were predominantly composed of the less stable polymorph, with a small amount of the more stable polymorph also present. Therefore, it is believed that aging induced the formation of crystals of the less stable polymorph in the drug concentrate, which then acted as seed material during the microprecipitation and homogenization steps such that the less stable polymorph was preferentially formed

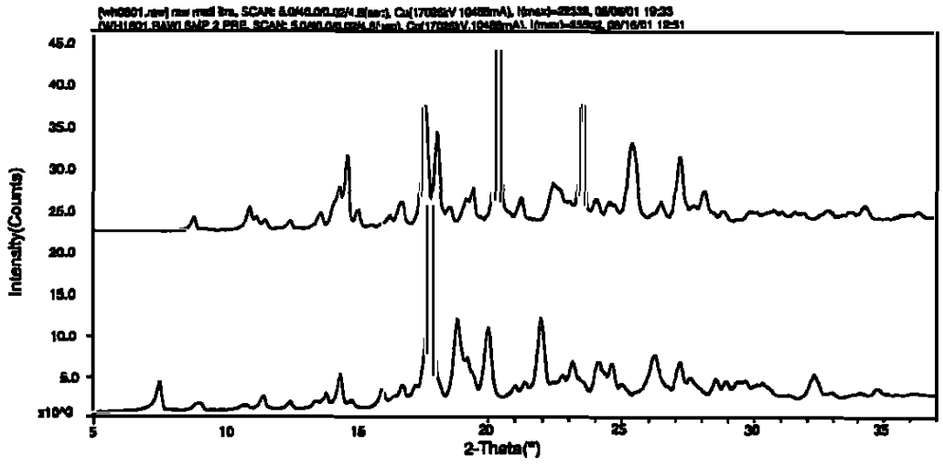


Figure 12 X-ray powder diffraction patterns for raw material itraconazole (top) and SMP-2-PRE (bottom) The raw material pattern has been shifted upward for clarity (Example 16)

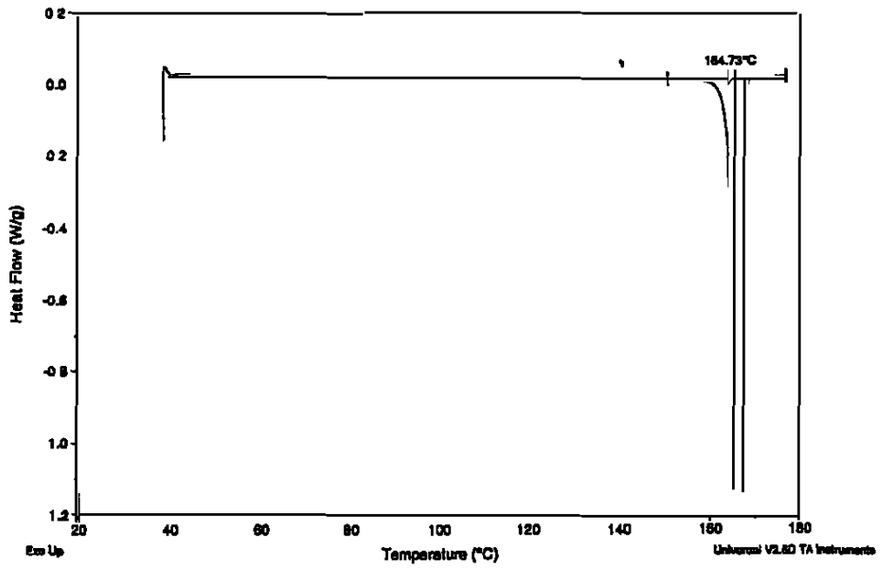


Figure 13a. DSC trace for raw material itraconazole (Example 16)

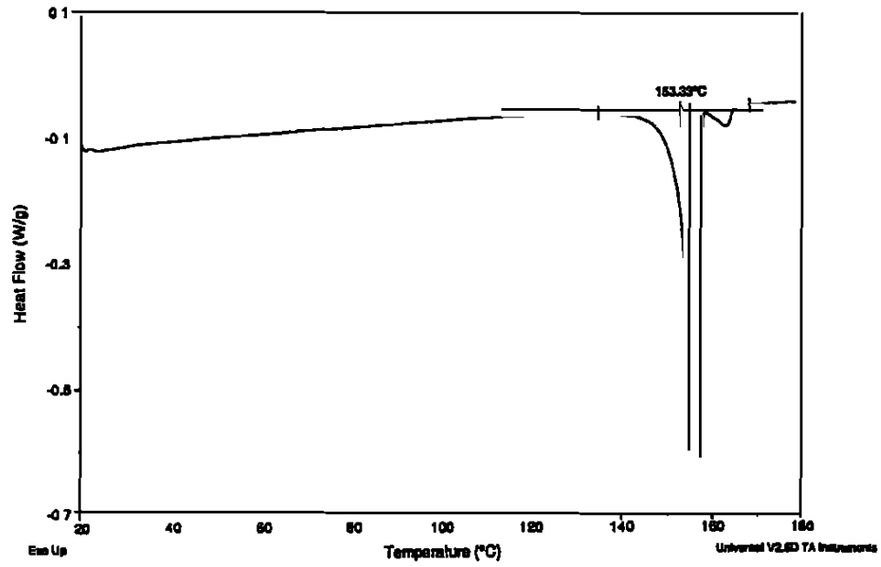


Figure 13b DSC trace for SMP 2-PRE (Example 16)

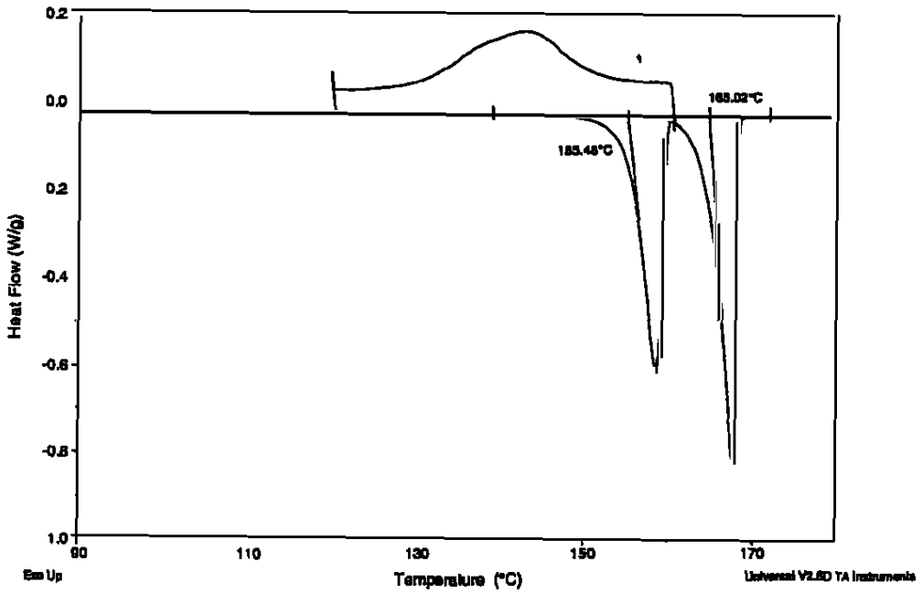


Figure 14 DSC trace for SMP-2-PRE showing the melt of the less stable polymorph upon heating to 160 °C, a recrystallization event upon cooling, and the subsequent melting of the more stable polymorph upon reheating to 180 °C (Example 16)

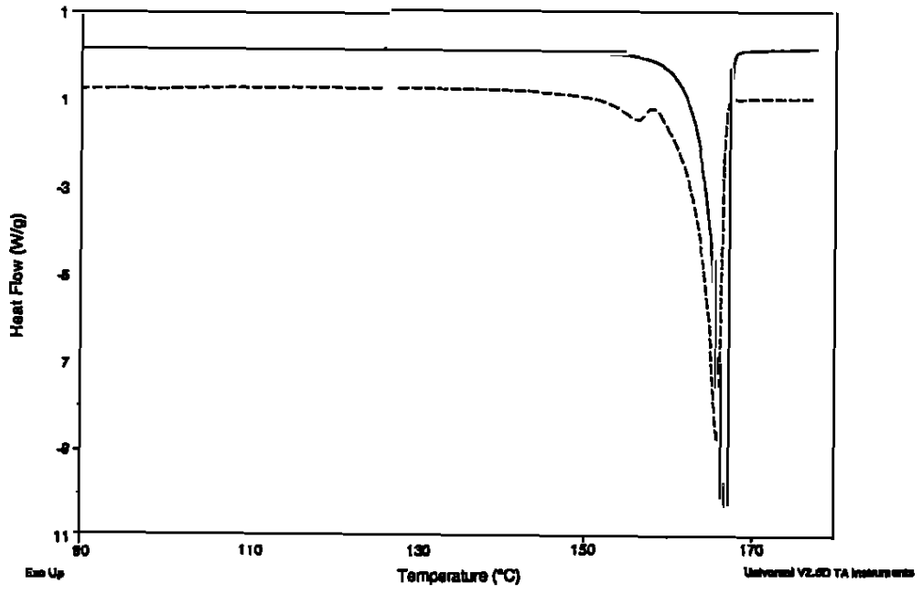


Figure 15 Comparison of SMP-2-PRE samples after homogenization Solid line = sample seeded with raw material itraconazole Dashed line = unseeded sample. The solid line has been shifted by 1 W/g for clarity (Example 16)

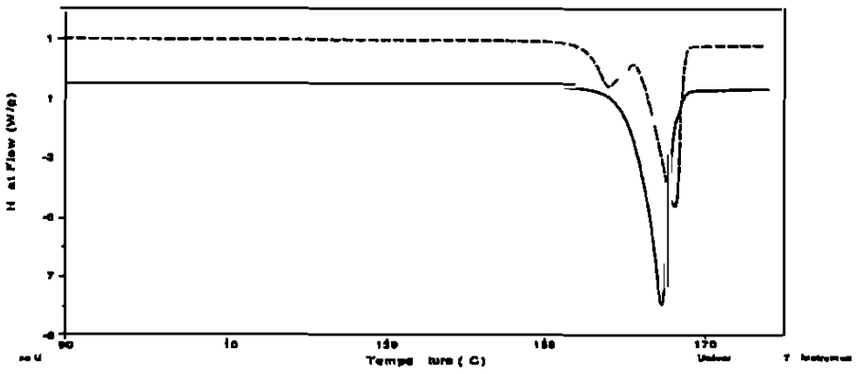


Figure 16 Effect of seeding during precipitation Dashed line = unseeded sample solid line = sample seeded with raw material itraconazole The unseeded trace (dashed line) has been shifted upward by 1 5 W/g for clarity (Example 17)

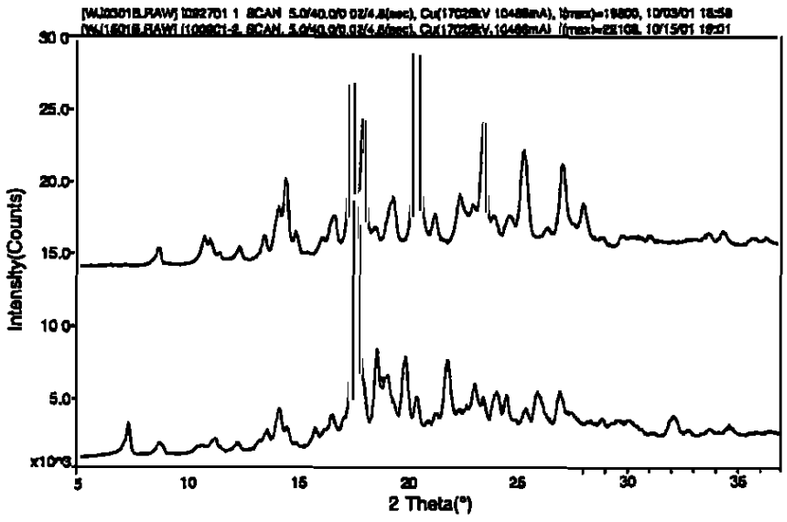


Figure 17 Effect of seeding the drug concentrate through aging Top x-ray diffraction pattern is for crystals prepared from fresh drug concentrate, and is consistent with the stable polymorph (see Figure 12, top) Bottom pattern is for crystals prepared from aged (seeded) drug concentrate, and is consistent with the metastable polymorph (see Figure 12, bottom) The top pattern has been shifted upward for clarity (Example 18)

While specific embodiments have been illustrated and described, numerous modifications come to mind without departing from the spirit of the invention and the scope of protection is only limited by the scope of the accompanying claims

CLAIMS

What is claimed is

1 A method for preparing submicron sized particles of an organic compound, the solubility of which is greater in a water-miscible first solvent than in a second solvent which is aqueous, the process comprising the steps of

(i) dissolving the organic compound in the water miscible first solvent to form a solution, the first solvent being selected from the group consisting of N-methyl-2 pyrrolidinone 2-pyrrolidone dimethyl sulfoxide dimethylacetamide, lactic acid, methanol ethanol isopropanol, 3-pentanol, n-propanol glycerol, butylene glycol, ethylene glycol, propylene glycol mono- and diacylated monoglycerides, dimethyl isosorbide, acetone, dimethylformamide, 1,4-dioxane, polyethylene glycol, polyethylene glycol esters, polyethylene glycol sorbitans, polyethylene glycol monoalkyl ethers, polypropylene glycol, polypropylene alginate, PPG-10 butanediol, PPG-10 methyl glucose ether PPG-20 methyl glucose ether, PPG-15 stearyl ether, propylene glycol dicaprylate, propylene glycol dicaprate, propylene glycol laurate,

(ii) mixing the solution with the second solvent to define a pre-suspension and

(iii) adding energy to the pre-suspension to form particles having an average effective particle size of less than about 2 μm.

2 The method of claim 1 further comprising the step of

mixing into the second solvent a first surface modifier selected from the group consisting of anionic surfactants, cationic surfactants, nonionic surfactants and surface active biological modifiers

3 The method of claim 2 wherein the nonionic surfactant is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene fatty acid esters, sorbitan esters, glycerol monostearate, polyethylene glycols, polypropylene glycols cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers, poloxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, polysaccharides starch starch derivatives, hydroxyethylstarch, polyvinyl alcohol, and polyvinylpyrrolidone

4 The method of claim 2 wherein the anionic surfactant is selected from the group consisting of potassium laurate, triethanolamine stearate, sodium lauryl sulfate, sodium dodecylsulfate alkyl polyoxyethylene sulfates, sodium alginate dioctyl sodium sulfosuccinate, phosphatidyl glycerol, phosphatidyl inositol, phosphatidylserine phosphatidic acid and their salts glyceryl esters, sodium carboxymethylcellulose, bile acids and their salts cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, and calcium carboxymethylcellulose

5 The method of claim 2 wherein the cationic surfactant is selected from the group consisting of quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium bromide,

chitosans and lauryldimethylbenzylammonium chloride

6 The method of claim 2 wherein the surface active biological modifiers are selected from the group consisting of albumin, casein, heparin, hirudin or other proteins

7 The method of claim 2 wherein the first solvent is N-methyl-2 pyrrolidinone

8 The method of claim 7 wherein the anionic surfactant is a copolymer of oxyethylene and oxypropylene

9 The method of claim 8 wherein the copolymer of oxyethylene and oxypropylene is a block copolymer

10 The method of claim 2 further comprising the step of mixing into the second solvent a second surface modifier

11 The method of claim 10 wherein the second surface modifier is selected from the group consisting of anionic surfactants cationic surfactants, nonionic surfactants and surface active biological modifiers

12 The method of claim 11 wherein the second surface modifier is a bile salt or a salt thereof

13 The method of claim 11 wherein the second surface modifier is selected from deoxycholic acid, glycocholic acid, glycodeoxycholic acid, taurocholic acid and salts of these acids

14 The method of claim 2 further comprising the step of adding a pH adjusting agent to the second solvent

15 The method of claim 14 wherein the pH adjusting agent is selected from the group consisting of sodium hydroxide hydrochloric acid, tris buffer citrate buffer acetate, lactate, and meglumine.

16 The method of claim 14 wherein the pH adjusting agent is added to the second solvent to bring the pH of the second solvent within the range of from about 3 to about 11

17 The method of claim 1 further comprising the step of
mixing into the solution a third surface modifier selected from the group consisting of anionic surfactants, canonic surfactants, nonionic surfactants and surface active biological modifiers

18 The method of claim 17 wherein the nonionic surfactant of the third surface modifier is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene sorbitan fatty acid esters polyoxyethylene fatty acid esters, sorbitan esters, glycerol monostearate polyethylene glycols polypropylene glycols cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers, polaxamines methylcellulose, hydroxycellulose, hydroxy propylcellulose hydroxy propylmethylcellulose noncrystalline cellulose, polysaccharides starch starch derivatives hydroxyethylstarch, polyvinyl alcohol, and polyvinylpyrrolidone

19 The method of claim 17 wherein the anionic surfactant of the third surface modifier is selected from the group consisting of potassium laurate, triethanolamine stearate, sodium lauryl

sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, phosphatidyl glycerol, phosphatidyl inositol phosphatidylserine phosphatidic acid and their salts, glyceryl esters, sodium carboxymethylcellulose bile acids and their salts and calcium carboxymethylcellulose

20 The method of claim 17 wherein the cationic surfactant of the third surface modifier is selected from the group consisting of quaternary ammonium compounds, benzalkonium chloride cetyltrimethylammonium bromide, chitosans and lauryldimethylbenzylammonium chloride

21 The method of claim 17 wherein the surface active biological modifiers are selected from the group consisting of albumin, casein, heparin, hirudin, or other proteins

22 The method of claim 17 wherein the first solvent is N methyl-2-pyrrolidinone

23 The method of claim 22 wherein the third surface modifier is a copolymer of oxyethylene and oxypropylene

24 The method of claim 23 wherein the copolymer of oxyethylene and oxypropylene is a block copolymer

25 The method of claim 17 further comprising the step of mixing into the solution a fourth surface modifier

26 The method of claim 25 wherein the fourth surface modifier is selected from the group consisting of anionic surfactants, cationic surfactants, nonionic surfactants and surface active biological modifiers

27 The method of claim 26 wherein the fourth surface modifier is a nonionic surfactant is selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene fatty acid esters, sorbitan esters, glycerol monostearate polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers polaxamines methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, polysaccharides, starch, starch derivatives, hydroxyethylstarch, polyvinyl alcohol, and polyvinylpyrrolidone

28 The method of claim 26 wherein the fourth surface modifier is an anionic surfactant selected from the group consisting of potassium laurate, triethanolamine stearate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, phosphatidyl glycerol, phosphatidyl inositol, phosphatidylserine, phosphatidic acid and their salts, glyceryl esters, sodium carboxymethylcellulose, bile acids and their salts and calcium carboxymethylcellulose

29 - - The method of claim 26 wherein the fourth surface modifier is a cationic surfactant selected from the group consisting of of quaternary ammonium compounds, benzalkonium chloride,

cetyltrimethylammonium bromide, chitosans and lauryldimethylbenzylammonium chloride

30 The method of claim 26 wherein the surface active biological modifiers are selected from the group consisting of albumin casein, heparin hirudin, or other proteins

31 The method of claim 17 further comprising the step of

mixing into the second solvent a fifth surface modifier selected from the group consisting of anionic surfactants cationic surfactants nonionic surfactants and surface active biological modifiers

32 The method of claim 31 wherein the fifth surface modifier is a nonionic surfactant selected from the group consisting of polyoxyethylene fatty alcohol ethers polyoxyethylene sorbitan fatty acid esters polyoxyethylene fatty acid esters sorbitan esters glycerol monostearate polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols polyoxyethylene-polyoxypropylene copolymers polaxamines, methylcellulose, hydroxycellulose hydroxy propylcellulose, hydroxy propylmethylcellulose noncrystalline cellulose, polysaccharides, starch, starch derivatives hydroxyethylstarch, polyvinyl alcohol, and polyvinylpyrrolidone

33 The method of claim 31 wherein the fifth surface modifier is an anionic surfactant selected from the group consisting of potassium laurate, triethanolamine stearate, sodium lauryl sulfate sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, phosphatidyl glycerol, phosphatidyl inositol, phosphatidylserine, phosphatidic acid and their salts glyceryl esters sodium carboxymethylcellulose bile acids and their salts and calcium carboxymethylcellulose

34 The method of claim 31 wherein the fifth surface modifier is a cationic surfactant selected from the group consisting of quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium bromide chitosans and lauryldimethylbenzylammonium chloride

35 The method of claim 31 wherein the surface active biological modifiers are selected from the group consisting of albumin casein heparin, hirudin, or other proteins

36 The method of claim 31 wherein the first solvent is N-methyl-2-pyrrolidinone

37 The method of claim 36 wherein the fifth surface modifier is a copolymer of oxyethylene and oxypropylene

38 The method of claim 37 wherein the copolymer of oxyethylene and oxypropylene is a block copolymer

39 The method of claim 31 further comprising the step of mixing into the solution a sixth surface modifier selected from the group consisting of anionic surfactants cationic surfactants nonionic surfactants and surface active biological modifiers

40 The method of claim 1 further comprising the step of

mixing into the second solvent a phospholipid

41 The method of claim 40 wherein the phospholipid is selected from natural phospholipids and synthetic phospholipids

42 The method of claim 40 wherein the phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol phosphatidylglycerol, phosphatidic acid, lysophospholipids egg phospholipid and soybean phospholipid

43 The method of claim 40 further comprising the step of mixing into the solution a seventh surface modifier selected from anionic surfactants cationic surfactants and non-ionic surfactants

44 The method of claim 43 wherein the nonionic surfactant of the seventh surface modifier is selected from the group consisting of polyoxyethylene fatty alcohol ethers polyoxyethylene sorbitan fatty acid esters polyoxyethylene fatty acid esters, sorbitan esters, glycerol monostearate, polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene polyoxypropylene copolymers polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, polysaccharides, starch, starch derivatives, hydroxyethylstarch polyvinyl alcohol, and polyvinylpyrrolidone

45 The method of claim 43 wherein the anionic surfactant of the seventh surface modifier is selected from the group consisting of potassium laurate, triethanolamine stearate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates sodium alginate, dioctyl sodium sulfosuccinate phosphatidyl glycerol, phosphatidyl inositol, phosphatidylserine, phosphatidic acid and their salts, glyceryl esters, sodium carboxymethylcellulose, bile acids and their salts and calcium carboxymethylcellulose

46 The method of claim 43 wherein the cationic surfactant of the seventh surface modifier is selected from the group consisting of quaternary ammonium compounds benzalkonium chloride, cetyltrimethylammonium bromide, chitosans and lauryldimethylbenzylammonium chloride

47 The method of claim 43 wherein the surface active biological modifiers are selected from the group consisting of albumin, casein heparin hirudin, or other proteins

48 The method of claim 43 wherein the seventh surface modifier is a bile acid or a salt thereof

49 The method of claim 43 wherein the first solvent is N-methyl-2-pyrrolidinone

50 The method of claim 49 further comprising the step of adding a phospholipid to the second solvent.

51 The method of claim 50 wherein the phospholipid is selected from natural phospholipids and synthetic phospholipids

52 ---The method of claim 50 wherein the phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol,

57/250

phosphatidylglycerol phosphatidic acid, lysophospholipids egg phospholipid and soybean phospholipid

53 The method of claim 50 further comprising the step of mixing into the solution an eighth surface modifier selected from anionic surfactants cationic surfactants and non ionic surfactants

54 The method of claim 53 wherein the nonionic surfactant of the eighth surface modifier is selected from the group consisting of polyoxyethylene fatty alcohol ethers sorbitan fatty acid esters, polyoxyethylene fatty acid esters sorbitan esters glycerol monostearate, polyethylene glycols, cetyl alcohol cetostearyl alcohol, stearyl alcohol poloxamers, polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose polyvinyl alcohol, and polyvinylpyrrolidone

55 The method of claim 53 wherein the anionic surfactant of the eighth surface modifier is selected from the group consisting of potassium laurate triethanolamine stearate sodium lauryl sulfate, sodium dodecylsulfate alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate phosphatidyl glycerol, phosphatidyl inositol, phosphatidylserine, phosphatidic acid and their salts, glyceryl esters, sodium carboxymethylcellulose, bile acids and their salts and calcium carboxymethylcellulose

56 The method of claim 53 wherein the cationic surfactant of the eighth surface modifier is selected from the group consisting of quaternary ammonium compounds benzalkonium chloride, cetyltrimethylammonium bromide chitosans and lauryldimethylbenzylammonium chloride

57 The method of claim 53 wherein the surface active biological modifiers are selected from the group consisting of albumin casein heparin, hirudin, or other proteins

58 The method of claim 53 wherein the eighth surface modifier is a bile acid or a salt thereof

59 The method of claim 58 wherein the first solvent is N-methyl-2-pyrrolidinone

60 A method for preparing submicron sized particles of an organic compound, the solubility of which is greater in a water-miscible first solvent than in a second solvent which is aqueous the process comprising the steps of

(i) dissolving the organic compound in the water-miscible first solvent to form a solution the first solvent being selected from the group consisting of N-methyl-2-pyrrolidinone 2-pyrrolidone dimethyl sulfoxide dimethylacetamide, lactic acid, methanol ethanol, isopropanol 3-pentanol n-propanol, glycerol, butylene glycol, ethylene glycol, propylene glycol, mono- and diacylated monoglycerides, dimethyl isosorbide, acetone dimethylformamide 1 4-dioxane,ethyl acetate propyl acetate, polyethylene glycol polyethylene glycol esters polyethylene glycol sorbitans polyethylene glycol monoalkyl ethers, polypropylene glycol, polypropylene alginate PPG-10 butanediol PPG-10 methyl glucose ether, PPG-20 methyl glucose ether, PPG-15 stearyl ether propylene glycol dicaprylate, propylene glycol dicaprate, propylene glycol laurate

(ii) mixing the solution with the second solvent to define a pre-suspension wherein the organic compound is in an amorphous form a semicrystalline form or in a supercooled liquid form as determined by DSC and having an average effective particle size and

(iii) annealing the pre suspension to form particles having essentially the same average effective particle size of the pre suspension and in a more stable form

61 The method of claim 60 wherein the annealing step includes the step of converting the particles of the presuspension to a crystalline form as determined by DSC

62 The method of claim 60 wherein the particles after the annealing step have a reduced tendency to aggregate into larger particles when compared to the particles of the pre suspension

63 The method of claim 60 wherein the particles of the presuspension have an average effective particle size of less than about 2 μm.

64 The method of claim 60 wherein the particles of the presuspension have an average effective particle size of from about 2 μm to about 50 nm

65 The method of claim 60 wherein the particles of the presuspension have an average effective particle size of less than about 400 nm

66 A method for preparing submicron sized particles of an organic compound, the solubility of which is greater in a water-miscible first solvent than in a second solvent which is aqueous, the process comprising the steps of

(i) dissolving the organic compound in the water miscible first solvent to form a solution, the first solvent being selected from the group consisting of N-methyl-2-pyrrolidinone 2-pyrrolidone, dimethyl sulfoxide, dimethylacetamide, lactic acid methanol, ethanol isopropanol, 3-pentanol n-propanol, glycerol, butylene glycol, ethylene glycol, propylene glycol, mono- and diacylated monoglycerides, dimethyl isosorbide, acetone dimethylformamide, 1,4-dioxane, polyethylene glycol, polyethylene glycol esters polyethylene glycol sorbitans polyethylene glycol monoalkyl ethers, polypropylene glycol, polypropylene alginate, PPG 10 butanediol, PPG 10 methyl glucose ether, PPG-20 methyl glucose ether, PPG-15 stearyl ether propylene glycol dicaprylate propylene glycol dicaprate, propylene glycol laurate,

(ii) mixing the solution with the second solvent to define a pre-suspension of particles in a friable form, and

(iii) adding energy to the pre-suspension to form particles having an average effective particle size of less than about 2 μm.

67 The method of claim 66 further comprising the step of

mixing into the second solvent a first surface modifier selected from the group consisting of anionic surfactants, cationic surfactants, nonionic surfactants and biological surface active molecules

68 The method of claim 67 wherein the first solvent is N-methyl 2-pyrrolidinone

69 The method of claim 68 wherein the anionic surfactant is a copolymer of oxyethylene and oxypropylene

70 The method of claim 69 wherein the copolymer of oxyethylene and oxypropylene is a block copolymer

71 The method of claim 67 further comprising the step of mixing into the second solvent a second surface modifier selected from the group consisting of anionic surfactants cationic surfactants, nonionic surfactants and biological surface active molecules

72 The method of claim 71 wherein the second surface modifier is a bile salt or a salt thereof

73 The method of claim 71 wherein the second surface modifier is selected from deoxycholic acid glycocholic acid, glycodeoxycholic acid, taurocholic acid and salts of these acids

74 The method of claim 67 further comprising the step of adding a pH adjusting agent to the second solvent

75 The method of claim 74 wherein the pH adjusting agent is selected from the group consisting of sodium hydroxide hydrochloric acid, tris buffer citrate buffer, acetate lactate and meglumine.

76 The method of claim 74 wherein the pH adjusting agent is added to the second solvent to bring the pH of the second solvent within the range of from about 3 to about11

77 The method of claim 66 further comprising the step of

mixing into the solution a third surface modifier selected from the group consisting of anionic surfactants, cationic surfactants, nonionic surfactants and biological surface active molecules

78 The method of claim 77 wherein the first solvent is N-methyl 2-pyrrolidinone

79 The method of claim 78 wherein the third surface modifier is a copolymer of oxyethylene and oxypropylene

80 The method of claim 79 wherein the copolymer of oxyethylene and oxypropylene is a block copolymer

81 The method of claim 77 further comprising the step of mixing into the solution a fourth surface modifier selected from the group consisting of anionic surfactants, cationic surfactants nonionic surfactants and biological surface active molecules

82 The method of claim 77 further comprising the step of

mixing into the second solvent a fifth surface modifier selected from the group consisting of anionic surfactants cationic surfactants nonionic surfactants and biological surface active molecules

83 The method of claim 82 wherein the first solvent is N-methyl-2 pyrrolidinone.

84 The method of claim 83 wherein the fifth surface modifier is a copolymer of oxyethylene and

oxypropylene

85 The method of claim 82 further comprising the step of mixing into the solution a sixth surface modifier selected from the group consisting of anionic surfactants cationic surfactants, nonionic surfactants and biological surface active molecules

86 The method of claim 66 further comprising the step of

mixing into the second solvent a phospholipid

87 The method of claim 86 wherein the phospholipid is selected from natural phospholipids and synthetic phospholipids

88 The method of claim 86 wherein the phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol phosphatidylglycerol, phosphatidic acid, lysophospholipids, egg phospholipid and soybean phospholipid

89 The method of claim 86 further comprising the step of mixing into the solution a seventh surface modifier selected from anionic surfactants, cationic surfactants, non-ionic surfactants and biological surface active molecules

90 The method of claim 89 wherein the seventh surface modifier is a bile acid or a salt thereof

91 The method of claim 90 wherein the first solvent is N-methyl 2-pyrrolidinone

92 The method of claim 91 further comprising the step of adding a phospholipid to the second solvent

93 The method of claim 92 wherein the phospholipid is selected from natural phospholipids and synthetic phospholipids

94 The method of claim 92 wherein the phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, lysophospholipids egg phospholipid and soybean phospholipid

95 The method of claim 92 further comprising the step of mixing into the solution an eighth surface modifier selected from anionic surfactants cationic surfactants, non-ionic surfactants and biological surface active molecules

96 The method of claim 95 wherein the eighth surface modifier is a bile acid or a salt thereof

97 The method of claim 96 wherein the first solvent is N-methyl-2-pyrrolidinone

98 A method for preparing a suspension of a pharmaceutically-active compound, the solubility of which is greater in a water-miscible first organic solvent than in a second solvent which is aqueous, the process comprising the steps of

(i) dissolving a first quantity of the pharmaceutically-active compound in the water-

miscible first organic solvent to form a first solution

(ii) mixing the first solution with the second solvent to precipitate the pharmaceutically active compound to create a presuspension, and

(iii) seeding the first solution or the second solvent prior to the or the presuspension after the mixing step

99 The method of claim 98 wherein the step of precipitating the pharmaceutically-active compound comprises the step of precipitating the compound in a form selected from the group consisting of a supercooled liquid, an amorphous particle a semicrystalline particle and a crystalline particle

100 The method of claim 99 further comprising the step of adding energy to the presuspension

101 The method of claim 100 wherein the adding-energy step comprises the step of subjecting the presuspension to high energy agitation

102 The method of claim 100 wherein the adding-energy step comprises the step of adding heat to the presuspension

103 The method of claim 100 wherein the energy-addition step comprises the step of exposing the presuspension to electromagnetic energy

104 The method of claim 103 wherein the step of exposing the presuspension to electromagnetic energy comprises the step of exposing the presuspension to a laser beam.

105 The method of claim 98 further comprising the step of forming a desired polymorph of the pharmaceutically active compound

106 The method of claim 105 wherein the step of seeding comprises the step of using a seed compound

107 The method of claim 105 wherein the seed compound is the desired polymorph of the pharmaceutically active compound

108 The method of claim 105 wherein the seed compound is a compound other than the desired polymorph of the pharmaceutically-active compound

109 The method of claim 108 wherein the seed compound is selected from the group consisting of an inert impurity and an organic compound with a structure similar to that of the desired polymorph.

110 The method of claim 105 wherein the seed compound is added to the first solution

111 The method of claim 105 wherein the seed compound is added to the second solvent.

112 The method of claim 105 wherein the seed compound is added to the presuspension

113 The method of claim 104 wherein the step of forming a desired polymorph comprises the step of forming a seed compound in the first solution

114 The method of claim 113 wherein the step of forming the seed compound in the first solution comprises the step of adding the pharmaceutically-active compound in sufficient quantity to exceed the solubility of the pharmaceutically-active compound in the first solvent to create a supersaturated solution

115 The method of claim 114 wherein the step of forming the seed compound in the first solution further comprises the step of treating the supersaturated solution

116 The method of claim 115 wherein the step of treating the supersaturated solution comprises the step of aging the supersaturated solution

117 The method of claim 116 wherein the seeding step comprises the step of using electromagnetic energy

118 The method of claim 117 wherein the electromagnetic energy is dynamic electromagnetic energy

119 The method of claim 117 wherein the electromagnetic energy is a laser beam

120 The method of claim 117 wherein the electromagnetic energy is radiation

121 The method of claim 98 wherein the step of seeding comprises the step of using a particle beam.

122 The method of claim 98 wherein the step of seeding comprises the step of using an electron beam.

123 The method of claim 98 wherein the step of seeding comprises using ultrasound

124 The method of claim 98 wherein the step of seeding comprises using a static electrical field

125 The method of claim 98 wherein the step of seeding comprises using a static magnetic field

126 The method of claim 98 further comprising the steps of forming particles having an average effective particle size less than about 2μm.

127 A composition of matter of a polymorphic pharmaceutically-active compound in a desired polymorphic form essentially free of an unspecified polymorphic form

128 The composition of claim 127 wherein the pharmaceutically-active compound is itraconazole

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 June 2003 (05 06.2003)

PCT

(10) International Publication Number
WO 03/045330 A2

(51) International Patent Classification[7] A61K

(21) International Application Number PCT/US02/38186

(22) International Filing Date
27 November 2002 (27 11 2002)

(25) Filing Language English

(26) Publication Language English

(30) Priority Data
09/995 403 27 November 2001 (27 11 2001) US

(71) Applicant *(for all designated States except US)* **THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS** [US/US] 352 Administration Building 506 South Wright Street Urbana IL 61801 (US)

(72) Inventors and
(75) Inventors/Applicants *(for US only)* **ONYUKSEL, Havat** [US/US] 4146 Claussen Avenue Western Springs IL 60558 (US) **RUBINSTEIN Israel** [IL/US] 2999 Lexington Lane Highland Park IL 60035 (US)

(74) Agent **WILLIAMS, Joseph, A., Jr** Marshall Gerstein & Borun 6300 Sears Tower 233 S Wacker Drive Chicago IL 60606 6357 (US)

(81) Designated States *(national)* AE, AG AL AM AT AU AZ, BA BB BG BR BY BZ, CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ, NO NZ, OM PH PL PT RO RU SC SD SL SG SI SK SL, TJ TM TN TR TT TZ, UA UG US UZ VC, VN YU ZA ZM ZW

(84) Designated States *(regional)* ARIPO patent (GH GM KE LS MW MZ SD SL, SZ TZ UG ZM ZW) Eurasian patent (AM AZ, BY KG KZ MD RU TJ TM) European patent (AT BE BG CH CY CZ DE DK EE ES FI FR GB GR, IE IT LU MC NL PT SE SK TR), OAPI patent (BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG)

Published
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations refer to the Guidance Notes on Codes and Abbreviations appearing at the beginning of each regular issue of the PCT Gazette

WO 03/045330 A2

(54) Title MATERIALS AND METHODS FOR MAKING IMPROVED MICELLE COMPOSITIONS

(57) Abstract Provided are methods of treatment of many different diseases and disorders using micelle and sterically stabilized crystalline compounds of the invention

bioactivity of the biologically active peptides in a manner which provides improvements in the efficacy and duration of the biological effects of the associated peptides Increased efficacy and duration of the biological effect is believed to result, at least in part, from interaction of the compound with the micelle in such a manner that the compound attains, and is maintained in, an active or more active conformation than the compound in an aqueous environment. The invention thus overcomes the problems associated with previous liposomal formulations, such as, but not limited to, uptake by the reticuloendothelial system, degradation of the compound, or delivery of the compound in an inactive conformation According to one aspect of the present invention, polyethylene-glycol (PEG) is covalently conjugated to DSPE and used to form polymeric micelles which are then passively loaded with VIP The PEG-DSPE forms micelles with a hydrophobic core consisting of distearoyl phosphatidylethanolamine (DSPE) fatty acid chains which is surrounded by a hydrophilic "shell" formed by the PEG polymer

According to one aspect of the invention, a method is provided for preparing a biologically active micelle product comprising one or more biologically active amphipathic compounds in association with a micelle, said method comprising the steps of a) mixing a combination of one or more lipids wherein said combination includes at least one lipid component covalently bonded to a water-soluble polymer; b) forming sterically stabilized micelles from said combination of lipids, and c) incubating micelles from step b) with one or more biologically active amphipathic compounds under conditions in which said compound become associated with said micelles from step b) in a more biologically active conformation as compared to the compound in an aqueous solution According to a further aspect of the invention, a biologically active micelle product may be produced by the coprecipitation of a biologically active amphipathic compound with lipids to form micelles with incubation not required Specifically, a method is provided of preparing a biologically active micelle product comprising one or more biologically active amphipathic compound in association with a micelle, said method comprising the steps of a) mixing one or more lipids wherein said combination includes at least one lipid component covalently bonded to a water-soluble polymer with a biologically active amphipathic compound, b) forming sterically stabilized micelles from the mixture of

glutamate-decarboxylase, GnRH / GL , keyhole limpet hemocyanin, leucin-enkephalin, mesotocin, methionin-enkephalin, neurotensin, peroxydase, somatostatin, substance P, vasopressin, and vasotocin The invention also contemplates modulators having enhanced bioactivity in association with micelles prepared by a method of the invention A particularly preferred peptide for use according to the invention is VIP In one aspect, micelles according to the invention are characterized by an average diameter of less than about 20 nm. According to one aspect of the invention the micelles further comprise calmodulin The biologically active peptide products of the invention may be utilized in a wide variety of therapeutic, diagnostic, cosmetic and organ, tissue and cell preservative uses wherein it is desired to deliver a high level of biologically active compound or to detect targeted delivery of the micelle product as will be described below

In another aspect, the invention provides methods for preparing a biologically active sterically stabilized crystalline product comprising one or more biologically active compounds which are insoluble in an aqueous solution, said method comprising the steps of a) dissolving in an organic solvent said biologically active compound(s) and one or more lipids wherein at least one lipid is conjugated to a water soluble polymer, b) removing the organic solvent to leave a dry film, c) hydrating the dry film with an aqueous solution, and d) forming a sterically stabilized crystalline product As used herein and in subsequently described crystalline products of the invention, "insoluble" is defined according to the U S Pharmacopeia National Formulary [USP 23, 1995, page 10], as requiring 10,000 or more parts of solvent for 1 part of solute The crystalline product of the method is essentially a micelle-encased aggregate of the insoluble compound which is densely packed and crystalized

In still another aspect, the invention provides methods for preparing a biologically active sterically stabilized crystalline product comprising one or more biologically active compounds which are insoluble in an aqueous solution, said method comprising the steps of a) dissolving in an organic solvent said biologically active compound and one or more lipids wherein at least one lipid is conjugated to a water soluble polymer, b) freeze-drying to remove the organic solvent, c) hydrating with an aqueous solution, and d) forming a sterically stabilized crystalline product

In still another aspect, the invention provides methods for preparing a biologically active sterically stabilized crystalline product comprising one or more

biologically active compounds which are insoluble in aqueous solution and one or more amphipathic targeting compounds, said method comprising the steps of a) dissolving in an organic solvent said biologically active compound(s) and one or more lipids wherein at least one lipid is conjugated to a water soluble polymer; b) removing the organic solvent to leave a dry film, c) hydrating the dry film with an aqueous solution, d) forming sterically stabilized crystalline products, e) combining said crystalline products with one or more targeting compounds, and f) incubating said crystalline products under conditions wherein the targeting compound(s) associates with said crystalline products, said targeting compound conjugated to a lipid of the micelle.

Methods of the invention for producing sterically stabilized crystalline products are amenable to the use of any compound that is insoluble in an aqueous solution Preferred insoluble compounds include, but are not limited to, progesterone, testosterone, estrogen, prednisolone, prednisone, 2,3 mercaptopropanol, amphotericin B, betulinic acid, camptothecin. diazepam, nystatin, propofol, cyclosporin A, doxorubicin, and paclitaxel (Taxol®), and tetramethyl NDGA In methods of the invention for producing sterically stabilized crystalline product further comprising one or more targeting compounds, any targeting compound that assumes or maintains a biologically active conformation when in association with the sterically stabilized crystalline product can be used. In a preferred embodiment, any of the amphipathic compounds as described above are utilized In a most preferred embodiment, the targeting compound is VIP or other member of the VIP/GRF family or proteins In other embodiments, the targeting compound can be heliospectins I or II or any member of the neuropeptide family such as neuropeptide Y (NPY), neuropeptide YY (NPYY), including neuropeptide fragments 2-36 and related fragments, ACTH, calcitonin, GAP (GnRII precursor molecule), glutamate-decarboxylase, keyhole limpet hemocyanin, leucin-enkephalin, mesotocin, methionin-enkephalin, neurotensin, peroxydase, somatostatin, substance P, vasopressin, and vasotocin

Composition comprising the biologically active micelle product of the invention include those wherein the biologically active amphipathic peptide, protein, fragment, analog, or modulators thereof has an activity selected from the group consisting of anti-oxidant activity, anti-pain, anti inflammatory, wound healing activity, anti-microbial, antibronchospasm, metabolic activity, anti-cancer activity

polymer and at least one biologically active compound is a member of the VIP/glucagon/secretin of IL-2 family of peptides including peptide fragments and analogs, and b) forming sterically stabilized crystalline products In one embodiment, medicaments of the invention include those wherein in the method of preparing the sterically stabilized crystalline compound, mixing in step (a) is carried out in an organic solvent, and forming crystalline products in step (b) is carried out in a process comprising the steps of (i) removing the organic solvent to leave a dry film, and (ii) hydrating the dry film with an aqueous solution, said method further comprising the steps of (c) contacting said crystalline products with one or more targeting compounds, and (d) incubating said crystalline products under conditions wherein the targeting compound(s) associates with said crystalline products In another aspect, medicaments according to the invention include those wherein in the method of preparing the sterically stabilized crystalline compound, forming in step(b) is carried out in the steps comprising (i) removing the organic solvent to leave a dry film and (ii) hydrating the dry film with an aqueous solution

In one embodiment, medicaments of the invention include micelle compositions or crystalline compounds wherein the water soluble polymer is polyethylene glycol (PEG) In another embodiment, medicaments of the invention include use of micelles having an average diameter of less than about 25 nm In still another embodiment, medicaments of the invention include micelle compositions or crystalline compounds wherein the combination of lipids consists of distearoyl-phosphatidylethanolamine covalently bonded to PEG (PEG DSPE)

The invention also provides a method of treating a pathology selected from the group consisting of immune disorders, inflammatory conditions, and cancer comprising the step of administering to an individual suffering from the pathology an amount of a micelle composition effective to ameliorate conditions associated with the pathology, said micelle composition prepared by a method of comprising the steps of

In still another aspect, the invention provides a method of treating a pathology selected from the group consisting of Hashimoto's thyroiditis, pernicious anemia, Addison s disease, diabetes systemic lupus erythematosus, dermatomyositis, Sjogren's syndrome, dermatomyositis, multiple sclerosis, myasthenia gravis, Reiter's syndrome, Graves disease, inflammatory bowel disease osteoarthritis rheumatoid

encapsulated agent SSM being more dynamic than liposomes may show superiority to SSL with respect to drug release Polymers of the invention may thus include any compounds known and routinely utilized in the art of sterically stabilized liposome (SSL) technology and technologies which are useful for increasing circulatory half-life for proteins, including for example polyvinyl alcohol, polylactic acid, polyglycolic acid, polyvinylpyrrolidone, polyacrylamide, polyglycerol, polyaxozlines, or synthetic lipids with polymeric headgroups The most preferred polymer of the invention is PEG at a molecular weight between 1000 and 5000 Preferred lipids for producing micelles according to the invention include distearoyl-phosphatidylethanolamine covalently bonded to PEG (PEG-DSPE) alone or in further combination with phosphatidylcholine (PC), and phosphatidylglycerol (PG) in further combination with cholesterol (Chol) and/or calmodulin

Methods of the invention for preparation of sterically stabilized micelle products or sterically stabilized crystalline products can be carried using various techniques In one aspect, micelle components are mixed in an organic solvent and the solvent is removed using either evaporation or lyophilization Removal of the organic solvent results in a lipid film, or cake, which is subsequently hydrated using an aqueous solution to permit formation of micelles The resulting micelles are mixed with an amphipathic compound of the invention whereby the amphipathic compound associates with the micelle and assumes a more favorable biologically active conformation

In a more simplified preparation technique, one or more lipids are mixed in an aqueous solution after which the lipids spontaneously form micelles The resulting micelles are mixed with an amphipathic compound which associates with the micelle products and assumes a more favorable biologically active conformation Preparing micelle products by this method is particularly amenable for large scale and safer preparation and requires a considerable shorter time frame than methods previously described The procedure is inherently safer in that use of organic solvents is eliminated

In methods of the invention for preparing sterically stabilized crystalline products, it is preferred that one or more lipid compounds are mixed in an organic solvent with one or more insoluble compounds The organic solvent is removed either by evaporation or lyophilization to provide a film, or cake The

Sterically stabilized crystalline products of the invention are particularly useful for administration of anti-cancer agents For example, crystalline products of the invention comprising paclitaxel (Taxol®) as the insoluble compound and VIP as the targeting agent can be targeted to breast cancer cells which are known to express higher levels of VIP receptor than normal breast cells, or express receptors with higher affinity of binding for VIP Paclitaxel (Taxol®) has been shown to selectively kill breast cancer cells

Cosmetic uses for the micelle and crystalline products of the invention include anti-aging , anti-wrinkling, and antioxidant activities, as well as use as a sunscreen.

The present invention is further illustrated by way of the following examples which used the following materials lipids L-α-egg yolk phosphatidylcholine type V-E in chloroform methanol (9 1) (Lot # 34H8395, and 75H8368), L-α-egg yolk phosphatidyl-D-α-Glycerol in chloroform methanol (98 2) (Lot # 72H8431, and 85H8395), and cholesterol (Lot #60H0476) from Sigma Chemical Co (St Louis, MO) Di-Palmitoyl-phosphatidyl choline (Lot #LP-04-01-112-187) from Sygenal Ltd (Switzerland) PEG-DSPE in lyophilized powder form (Lot # 180PHG2PK-26) from Avanti Polar Lipids Inc (Alabaster, AL) Peptides VIP (Lot # K02012A1, F02018A1, and K02018A1), VIP fragment 1-12 (Lot # H05009T1), VIP fragment 10-28 (Lot # NB0222), and Vasopressin (Lot # SD1051A) from American Peptide Co (Sunnyvale CA) Other bio-products Bovine Brain Calmodulin (Lot # B10537) from Calbiochem Intl (La Jolla, CA) ELISA assay kit (Lot # 976605) from Peninsula Laboratories (Belmont, CA) Various chemicals trehalose (Lot # 43H7060), 2,4-diaminophenol (amidol, Lot # 74H3652), ammonium molybdate (Lot # 42H3506), sodium bisulfite (Lot # 41H09432), HEPES (Lot # 43H5720), and sodium chloride (Lot # 22H0724) from Sigma Chemicals Co (St Louis, MO) Sodium dodecyl sulfate (Lot # 11120KX) from Aldrich Chemical Co , Inc Perchloric acid 70 % (Lot # 945567) chloroform HPLC grade (Lot # 902521) and potassium phosphate monobasic (Lot # 914723) from Fisher Sci (Pittsburgh, PA) "Inflammation" as used herein refers to a localized, protective response elicited by injury or destruction of tissues, which serves to destroy, dilute or wall off (sequester) both the injurious agent and the injured tissue Inflammation is notably associated with influx of leukocytes and or neutrophil chemotaxis Inflammation may

US005916596A

United States Patent [19]

Desai et al

[11] Patent Number 5,916,596

[45] Date of Patent Jun 29, 1999

70
263

[54] PROTEIN STABILIZED PHARMACOLOGICALLY ACTIVE AGENTS, METHODS FOR THE PREPARATION THEREOF AND METHODS FOR THE USE THEREOF

[75] Inventors Neil P Desai Los Angeles Chunlin Tao Beverly Hills Andrew Yang, Rosemead Leslie Loute Montebello Tianli Zheng Zhiwen Yao both of Culver City Patrick Soon Shiong Los Angeles, all of Calif Shlomo Magdassi Jerusalem Israel

[73] Assignee Vivorx Pharmaceuticals, Inc Santa Monica Calif

[21] Appl No 08/720,756

[22] Filed Oct 1, 1996

Related U S Application Data

[60] Continuation in part of application No 08/412 726 Mar 29 1995 Pat No 5,560 933 which is a division of application No 08/023,698 Feb 22 1993 Pat No 5 439,686

[51] Int Cl [6] A61K 9/14

[52] U S Cl 424/489 424/450 424/465 424 451 424/439

[58] Field of Search 424/489 422 424 423 475 9 1 9 3 9 32, 450 400

[56] References Cited

U S PATENT DOCUMENTS

3 959 457 5/1976 Speaker et al
4 073,943 2/1978 Wretlind et al
4 247 406 1/1981 Widder et al
4 572,203 2/1986 Feinstein

(List continued on next page)

FOREIGN PATENT DOCUMENTS

0 129 619 A1 1/1985 European Pat Off
0 295 941 A2 12/1988 European Pat Off
0 391 518 A2 2/1990 European Pat Off

(List continued on next page)

OTHER PUBLICATIONS

Burgess et al "Potential use of albumin microspheres as a drug delivery system I Preparation and in vitro release of steroids, *International Journal of Pharmaceutics* 39 129–136 (1987)

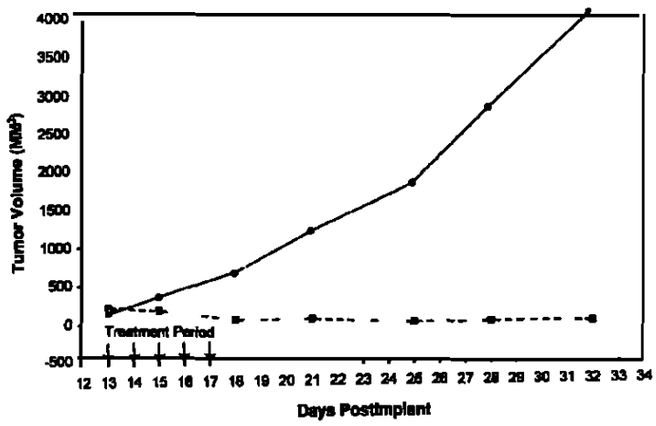
(List continued on next page)

Primary Examiner—Neil S Levy
Assistant Examiner—William L Benston, Jr
Attorney, Agent or Firm—Gray Cary Ware & Freidenrich Stephen E Reiter

[57] ABSTRACT

In accordance with the present invention there are provided compositions and methods useful for the in vivo delivery of substantially water insoluble pharmacologically active agents (such as the anticancer drug paclitaxel) in which the pharmacologically active agent is delivered in the form of suspended particles coated with protein (which acts as a stabilizing agent) In particular protein and pharmacologically active agent in a biocompatible dispersing medium are subjected to high shear in the absence of any conventional surfactants and also in the absence of any polymeric core material for the particles. The procedure yields particles with a diameter of less than about 1 micron The use of specific composition and preparation conditions (e g addition of a polar solvent to the organic phase) and careful selection of the proper organic phase and phase fraction enables the reproducible production of unusually small nanoparticles of less than 200 nm diameter which can be sterile filtered The particulate system produced according to the invention can be converted into a redispersible dry powder comprising nanoparticles of water insoluble drug coated with a protein and free protein to which molecules of the pharmacological agent are bound This results in a unique delivery system in which part of the pharmacologically active agent is readily bioavailable (in the form of molecules bound to the protein) and part of the agent is present within particles without any polymeric matrix therein

31 Claims, 2 Drawing Sheets



Pharm Sci 85 530 (1996) The process is based on dissolv ing the drug (e g indomethacin) and the polymer (poly caprolactone) in methylene chloride and acetone and then pouring the solution into an aqueous phase containing a surfactant (Poloxamer 188) to yield submicron size par ticles (216 nm) However the process is performed at solvent concentrations at which no emulsion is formed

BRIEF DESCRIPTION OI THE INVENTION

Thus it is an object of this invention to deliver pharma cologically active agents (e g taxol taxane Taxotere and the like) in unmodified form in a composition that does not cause allergic reactions due to the presence of added emul sifiers and solubilizing agents as are currently employed in drug delivery

It is a further object of the present invention to deliver pharmacologically active agents in a composition of micro particles or nanoparticles, optionally suspended in a suitable biocompatible liquid

It is yet another object of the present invention to provide a method for the formation of submicron particles (nanoparticles) of pharmacologically active agents by a solvent evaporation technique from an oil in water emulsion using proteins as stabilizing agents in the absence of any conventional surfactants and in the absence of any poly meric core material

These and other objects of the invention will become apparent upon review of the specification and claims

In accordance with the present invention we have dis covered that substantially water insoluble pharmacologi cally active agents can be delivered in the form of micro particles or nanoparticles that are suitable for parenteral administration in aqueous suspension This mode of delivery obviates the necessity for administration of substantially water insoluble pharmacologically active agents (e g taxol) in an emulsion containing for example ethanol and poly ethoxylated castor oil diluted in normal saline (see for example Norton et al in *Abstracts of the 2nd National Cancer Institute Workshop on Taxol & Taxus* Sep 23–24 1992) A disadvantage of such known composition is their propensity to produce allergic side effects

Thus in accordance with the present invention here are provided methods for the formation of nanoparticles of pharmacologically active agents by a solvent evaporation technique from an oil in water emulsion prepared under conditions of high shear forces (e g sonication high pres sure homogenization or the like) without the use of any conventional surfactants, and without the use of any poly meric core material to form the matrix of the nanoparticle Instead proteins (e g human serum albumin) are employed as a stabilizing agent

The invention further provides a method for the repro ducible formation of unusually small nanoparticles (less than 200 nm diameter) which can be sterile filtered through a 0 22 micron filter This is achieved by addition of a water soluble solvent (e g ethanol) to the organic phase and by carefully selecting the type of organic phase the phase fraction and the drug concentration in the organic phase The ability to form nanoparticles of a size that is filterable by 0 22 micron filters is of great importance and significance since formulations which contain a significant amount of any protein (e g albumin) cannot be sterilized by conven tional methods such as autoclaving due to the heat coagu lation of the protein

In accordance with another embodiment of the present invention we have developed compositions useful for in vivo delivery of substantially water insoluble pharmacologi cally active agents Invention compositions comprise sub stantially water insoluble pharmacologically active agents (as a solid or liquid) contained within a polymeric shell The polymeric shell is a crosslinked biocompatible polymer The polymeric shell containing substantially water insoluble pharmacologically active agents therein can then be sus pended in a biocompatible aqueous liquid for administra tion

The invention further provides a drug delivery system in which part of the molecules of pharmacologically active agent are bound to the protein (e g human serum albumin) and are therefore immediately bioavailable upon adminis tration to a mammal The other portion of the pharmaco logically active agent is contained within nanoparticles coated by protein The nanoparticles containing the phar macologically active agent are present as a pure active component without dilution by any polymeric matrix

A large number of conventional pharmacologically active agents circulate in the blood stream bound to carrier proteins (through hydrophobic or ionic interactions) of which the most common example is serum albumin Invention meth ods and compositions produced thereby provide for a phar macologically active agent that is pre-bound to a protein (through hydrophobic or ionic interactions) prior to admin istration

The present disclosure demonstrates both of the above described modes of bioavailability for Taxol (Paclitaxel) an anticancer drug capable of binding to human serum albumin (see for example Kumar et al in *Research Communica tions in Chemical Pathology and Pharmacology* 80 337 (1993)) The high concentration of albumin in invention particles, compared to Taxol provides a significant amount of the drug in the form of molecules bound to albumin which is also the natural carrier of the drug in the blood stream

In addition advantage is taken of the capability of human serum albumin to bind Taxol as well as other drugs which enhances the capability of Taxol to absorb on the surface of the particles Since albumin is present on the colloidal drug particles (formed upon removal of the organic solvent) formation of a colloidal dispersion which is stable for prolonged periods is facilitated due to a combination of electrical repulsion and steric stabilization

In accordance with the present invention there are also provided submicron particles in powder form which can easily be reconstituted in water or saline The powder is obtained after removal of water by lyophilization Human serum albumin serves as the structural component of inven tion nanoparticles and also as a cryoprotectant and recon stitution aid The preparation of particles filterable through a 0 22 micron filter according to the invention method as described herein followed by drying or lyophilization pro duces a sterile solid formulation useful for intravenous injection

The invention provides, in a particular aspect a compo sition of anti-cancer drugs e g Taxol in the form of nanoparticles in a liquid dispersion or as a solid which can be easily reconstituted for administration Due to specific properties of certain drugs, e g Taxol such compositions can not be obtained by conventional solvent evaporation methods that rely on the use of surfactants In the presence of various surfactants very large drug crystals (e g size of about 5 microns to several hundred microns) are formed within a few minutes of storage after the preparation process The size of such crystals is typically much greater than the allowed size for intravenous injection

in saline and administered to a first group of 3 Sprague Dawley rats by intravenous bolus. A second group of 3 rats were given Sandimmune I V which contains Cremaphor/Ethanol after dilution in saline Each group received the same dose of 2 5 mg/kg Blood samples were taken at times 0 5 15 30 (minutes) 1 2 4 8 24 36 and 48 (hours) Levels of cyclosporine in the blood were assayed by HPLC and typical PK parameters were determined The PK curves showed typical decay over time as follows

	Decay Over Time	
	AUC mg hr/ml	Cmax, ng/ml
Capsorine I V	12,228	2,803
Sandimmune I V	7 791	2,606

In addition due to toxicity of the Sandimmune I V formulation 2 of 3 rats in that group died within 4 hours after dosing Thus the nanoparticle formulation (Capsorine I V) according to the present invention shows a greater AUC and no toxicity compared to the commercially available formulation (Sandimmune I V)

LXAMPI L 25

Pharmacokinetic (PK) Data for Cyclosporine Nanodroplets (Capsorine Oral) Following Oral Administration Comparison with Neoral (Currently Marketed I ormulation by Sandoz)

Nanodroplets of cyclosporine prepared above were administered in orange juice to a first group of 3 Sprague Dawley rats by oral gavage A second group of 3 rats were given Neoral a commercially available microemulsion formulation containing emulsifiers after dilution in orange juice also by oral gavage Each group received the same dose of 12 mg/kg in an identical volume of orange juice Blood samples were taken at times 0 5 15 30 (minutes) 1 2 4 8 24 36 and 48 (hours) Levels of cyclosporine in the blood were assayed by HPI C and typical PK parameters were determined The PK curves showed typical decay over time as follows

	Decay Over Time	
	AUC ng-hr/ml	Cmax ng/ml
Capsorine Oral	1 195	887
Neoral	1,213	690

Thus the nanodroplet formulation (Capsorine Oral) of the present invention shows a similar PK behavior to the commercially available formulation (Neoral)

While the invention has been described in detail with reference to certain preferred embodiments thereof it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed

That which is claimed is

1 A method for the preparation of a substantially water insoluble pharmacologically active agent for in vivo delivery said method comprising

subjecting a mixture comprising

an organic phase containing said pharmacologically active agent dispersed therein and

aqueous medium containing biocompatible polymer

wherein said mixture contains substantially no surfactants, to high shear conditions in a high pressure homogenizer at a pressure in the range of about 3,000 up to 30,000 psi

2 A method according to claim 1 further comprising removing, said organic phase from said mixture

3 A method according to claim 1 further comprising removing said aqueous phase from said mixture

4 A method according to claim 1 wherein said substantially water insoluble pharmacologically active agent is selected from a pharmaceutically active agent a diagnostic agent or an agent of nutritional value

5 A method according to claim 4 wherein said pharmaceutically active agent is selected from the group consisting of analgesics/antipyretics anesthetics antiasthamatics antibiotics, antidepressants antidiabetics, antifungal agents antihypertensive agents anti inflammatories antineoplastics, antianxiety agents immunosuppressive agents antimigraine agents sedatives/hypnotics antiangmal agents antipsychotic agents antimanic agents antiarrhythmics antiarthritic agents antigout agents anticoagulants, thrombolytic agents antifibrinolytic agents hemorheologic agents antiplatelet agents anticonvulsants antiparkinson agents, antihistamines/antipruritics, agents useful for calcium regulation antibacterial agents, antiviral agents, antimicrobials, anti infectives bronchodialators hormones hypoglycemic agents, hypolipidemic agents proteins nucleic acids agents useful for erythropoiesis stimulation antiulcer/antireflux agents antinauseants/antiemetics, oil soluble vitamins as well as mitotane visadine halonitrosoureas, anthrocyclines and ellipticine

6 A method according to claim 4 wherein said pharmaceutically active agent is an antineoplastic selected from adriamycin cyclophosphamide actinomycin bleomycin duanorubicin doxorubicin epirubicin mitomycin methotrexate fluorouracil carboplatin carmustine (BCNU) methyl CCNU cisplatin etoposide interferon camptothecin and derivatives thereof phenesterine paclitaxel and derivatives thereof taxotere and derivatives thereof vinblastine vincristine tamoxifen etoposide or piposulfan

7 A method according to claim 4 wherein said pharmaceutically active agent is an immunosuppressive agent selected from cyclosporine azathioprine mizoribine or I K506 (tacrolimus)

8 A method according to claim 4 wherein said diagnostic agent is selected from ultrasound contrast agents radiocontrast agents, or magnetic contrast agents

9 A method according to claim 4 wherein said agent of nutritional value is selected from amino acids, sugars proteins carbohydrates fat soluble vitamins or fat or combinations of any two or more thereof

10 A method according to claim 1 wherein said organic phase has a boiling point of no greater than about 200° C

11 A method according to claim 10 wherein said organic phase is selected from soybean oil coconut oil olive oil safflower oil cotton seed oil sesame oil orange oil limonene oil, aliphatic cycloaliphatic or aromatic hydrocarbons having 4–30 carbon atoms aliphatic or aromatic alcohols having 2–30 carbon atoms, aliphatic or aromatic esters having 2–30 carbon atoms alkyl aryl or cyclic ethers having 2–30 carbon atoms, alkyl or aryl halides having 1–30 carbon atoms optionally having more than one halogen substituent ketones having 3–30 carbon atoms polyalkylene glycol or combinations of any two or more thereof

12 A method according to claim 10 wherein said organic phase comprises a mixture of a substantially water immiscible organic solvent and a water soluble organic solvent

13 A method according to claim 1 wherein said biocompatible polymer is a naturally occurring polymer a synthetic polymer or a combination thereof

14 A method according to claim 1 wherein said biocompatible polymer is capable of being crosslinked by disulfide bonds

15 A method according to claim 13 wherein said naturally occurring polymers are selected from proteins, peptides polynucleic acids polysaccharides proteoglycans or lipoproteins

16 A method according to claim 13 wherein said synthetic polymers are selected from synthetic polyamino acids containing cysteine residues and/or disulfide group polyvinyl alcohol modified to contain free sulfhydryl groups and/or disulfide groups, polyhydroxyethyl methacrylate modified to contain free sulfhydryl groups and/or disulfide groups polyacrylic acid modified to contain free sulfhydryl groups and/or disulfide groups, polyethyloxazoline modified to contain free sulfhydryl groups and/or disulfide groups polyacrylamide modified to contain free sulfhydryl groups and/or disulfide groups polyvinyl pyrrolidinone modified to contain free sulfhydryl groups and/or disulfide groups, polyalkylene glycols modified to contain free sulfhydryl groups and/or disulfide groups polylactides polyglycolides polycaprolactones or copolymers thereof modified to contain free sulfhydryl groups and/or disulfide groups, as well as mixtures of any two or more thereof

17 A method according to claim 1 wherein said high shear conditions comprise contacting said organic phase and said aqueous medium in a high pressure homogenizer at a pressure in the range of about 6 000 up to 25 000 psi

18 A method according to claim 1 wherein said biocompatible polymer is the protein albumin

19 A method according to claim 1 wherein said aqueous medium is selected from water buffered aqueous media saline buffered saline solutions of amino acids solutions of sugars solutions of vitamins, solutions of carbohydrates, or combinations of any two or more thereof

20 A method according to claim 1 wherein said high shear conditions produce particles comprising said pharmacologically active agent coated with said biocompatible polymer

21 A method according to claim 20 wherein said particles have an average diameter of less than 1 micron

22 A method according to claim 20 wherein said particles have an average diameter of less than 200 nm

23 A method according to claim 22 wherein said mixture is sterile filtered

24 A method according to claim 20 wherein said particles are amorphous, crystalline or a mixture thereof

25 A method according to claim 24 wherein said particles are substantially amorphous

26 A method for the preparation of a substantially water insoluble pharmacologically active agent for in vivo delivery in the form of sterile filterable particles said method comprising

subjecting a mixture comprising

an organic phase containing said pharmacologically active agent dispersed therein wherein said organic phase comprises a mixture of a substantially water immiscible organic solvent and a water soluble organic solvent and

aqueous medium containing biocompatible polymer

wherein said mixture contains substantially no surfactants, to high shear conditions in a high pressure homogenizer at a pressure in the range of about 3 000 up to 30,000 psi

27 A method according to claim 26 further comprising removing said organic phase from said mixture

28 A method according to claim 26 further comprising filtering said mixture through a 0 22 micron filter

29 A method according to claim 26 further comprising removing said aqueous phase from said mixture

30 A method according to claim 26 wherein said high shear conditions produce amorphous particles crystalline particles or a mixture thereof

31 A method according to claim 30 wherein said particles are substantially amorphous

* * * * *

Industria y Comercio
SUPERINTENDENCIA

2020
Bogotá, D C ,

Doctor
Emilio Ferrero
Apoderado

Oficio N° 10953

ASUNTO	Radicacion PCT N°	06-42082
	Tramite	011
	Evento	379
	Actuacion	440
	Folios	090

Con fundamento en lo previsto por el articulo 43 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica que la oposicion presentada por el doctor Jose Luis Reyes Villamizar, en su calidad de apoderado de Tecnoquimicas S A , reune los requisitos formales exigidos por la ley al momento de su presentacion

En cumplimiento de lo dispuesto en la citada norma, se le concede el termino de sesenta (60) dias habiles siguientes a la fecha de notificación del presente oficio para que haga valer sus argumentaciones y presente pruebas

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5 2, literal e), del capitulo quinto del titulo primero de la Circular Unica N° 10 de 2001

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

El antenor oficio fue notificado por fijación en lista de fecha, 07 SET. 2007

202021

Sede Centro Carrera 13 No 27 00 Pisos 2 5 7 y 10
Sede CAN Tr 40A No 38 50 Conmutador 3820840
Fax 382 26 95 Linea 9800-910 165
Web www sic gov co e mail info@sic gov co
Bogotá D C Colombia