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Señor Director
DIRECCION DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

Trámite : 011
Evento : 378
Actuación : 444

Referencia	:	Expediente **No. 06.042.082**
Asunto	:	Solicitud de Patente para: **MÉTODO PARA PREPARAR PARTÍCULAS SUBMICRÓNIAS DE PACLITAXEL**
Solicitante	:	**BAXTER INTERNATIONAL INC.**
Fecha de Solicitud	:	04 de Mayo de 2006

1. Yo, Luz Clemencia de PAEZ, obrando como apoderada del solicitante en el asunto antes identificado, de acuerdo con la sustitución efectuada a mi nombre por el Doctor Emilio Ferrero, me refiero al Oficio No. **20173,** notificado por fijación en lista del 23 de Diciembre de 2011.

2. Mediante el Oficio No. **20173** se puso en conocimiento del solicitante el informe del examen de fondo que contiene las observaciones hechas por su Despacho sobre la presente solicitud de patente.

3. <u>De la solicitud de patente</u>

El Examinador considera que la reivindicación 1 carece de concisión, por cuanto define la materia reivindicada en términos de expresiones generales que no limitan el alcance de la materia reclamada. De la misma manera, indica que no se especifican las proporciones de los excipientes y activos en la formulación.

Adicionalmente, el Examinador sugiere especificar los métodos utilizados en el proceso reclamado para llevar a cabo las etapas de homogenización de la suspensión, y remoción de la fase líquida de la suspensión.

Por otro lado, se menciona que las reivindicaciones 29 y 30 caracterizan el método por la vía de administración del producto, debiendo estar el método caracterizado por el conjunto de etapas y condiciones que permiten lograr el producto deseado.

Finalmente, el Examinador considera que la reivindicación 33 debe redactarse nuevamente para caracterizar el compuesto farmacéutico en términos de los elementos estructurales que lo componen.

4. Nivel Inventivo

Para el análisis de patentabilidad, el Examinador ha citado las anterioridades D1: WO 99/059550, "*Novel particulate formulations*", y D2: WO 2002/055059, "*Method for preparing submicron particle suspensions*".

De esta manera, el Examinador indica que una persona del oficio normalmente versada en la materia, incluiría paclitaxel, siendo este un compuesto orgánico de baja solubilidad en agua, de acuerdo a lo enseñado en D1, en el procedimiento para preparar partículas de tamaño submicrónico de un compuesto orgánico de D2; razón por la cual no se puede atribuir altura inventiva a las reivindicaciones 1 a 33.

5. Respuesta a las objeciones

De este modo y con el objeto de atender las objeciones arriba mencionadas, se pone de presente al Despacho las siguientes modificaciones, aclaraciones y argumentos:

5.1. Modificación a una Solicitud en Trámite

De acuerdo con el artículo 34 de la Decisión 486 de la Comisión de la Comunidad Andina, donde se establece que la modificación de una solicitud es posible en cualquier momento del trámite mientras no implique una ampliación de la solicitud inicial, me permito presentar un nuevo pliego reivindicatorio conformado por 30 reivindicaciones, en el cual:

La reivindicación 1 ha sido modificada para especificar que el paclitaxel es precipitado, de acuerdo a lo demostrado en el ejemplo 19 de la presente solicitud (véase las páginas 59 y 60 de la memoria descriptiva). Así mismo, la nueva reivindicación 26 ahora indica las operaciones mediante las cuales se puede lograr la remoción de la fase líquida, específicamente a través de evaporación, evaporación rotativa, liofilización, secado-congelado, diafiltración, centrifugación, fraccionamiento de campo de fuerza, filtración de alta presión y osmosis reversa. Esta última modificación se basa en lo contemplado en la anterior reivindicación 27.

Por otro lado, el Examinador considera que la reivindicación 1 no es concisa, toda vez que no es posible definir el alcance de la materia reclamada al identificar los elementos constitutivos "primer solvente", "segundo solvente", "modificador de superficie", "fosfolípido", "polímero hidrofílico", "surfactantes aniónicos", "surfactantes catiónicos", "surfactantes no-iónicos" y "modificadores biológicos de superficie activa" por sus propiedades. Igualmente, indica que no se especifican las proporciones de los excipientes e ingredientes activos en la formulación.

No obstante, resulta necesario aclarar que el reconocimiento de estos compuestos se considera como un trabajo de rutina para un experto medio en la materia, ya que éste se encuentra en capacidad de utilizar sus conocimientos para la interpretación de dichos términos en composiciones farmacéuticas y en su elaboración, a partir de lo divulgado en la descripción de la presente solicitud. Como soporte de lo anterior, se trae a colación el artículo 51 de la Decisión 486 de la Comisión de la Comunidad Andina, el cual menciona:

> ***Artículo** 51.- El alcance de la protección conferida por la patente estará determinado por el tenor de las reivindicaciones. La descripción y los dibujos, o en su caso, el material biológico depositado, servirán para interpretarlas.*

Así las cosas, la memoria descriptiva, específicamente los segmentos de texto comprendidos entre la página 16, línea 18 a la página 17, línea 14; página 17, líneas 16 a 18; página 17, línea 28, a la página 18, línea 1; página 18, líneas 2 a 9 y líneas 13 a 21; página 18, líneas 10 a 13; página 19, líneas 14 a 19; página 18, líneas 22 a 27; página 19, líneas 10 a 27; y página 19, línea 28 a la página 20, línea 2; sirven para interpretar las definiciones de los compuestos primer y segundo solvente, modificadores de superficie, surfactantes aniónicos, surfactantes catiónicos, fosfolípidos, polímeros hidrofílicos, surfactantes no-iónicos, y modificadores biológicos de superficie activa, respectivamente. De igual manera, la descripción indica las diversas proporciones de los ingredientes activos y excipientes que pueden ser utilizados, como puede ser observado en la página 17, líneas 22 a 24, página 31, líneas 16 a 18, así como en la página 20, líneas 9 a 24.

Aún más, el método de preparación reclamado requiere que la solubilidad del paclitaxel o sus compuestos derivados sea mayor en un primer solvente miscible en agua que en un segundo solvente acuoso. Lo anterior, por cuanto dicho diferencial de solubilidad permite la formación de las partículas del ingrediente activo, por lo que se esperaría que una persona del oficio normalmente versada en la materia puede reconocer que otras combinaciones de solventes que presenten un diferencial de solubilidad como el especificado en la presente solicitud puedan ser empleadas para la ejecución de la invención bajo estudio.

De igual manera, la persona del oficio normalmente versada en la materia está en la capacidad de reconocer los posibles compuestos a ser usados como un segundo modificador de superficie (surfactantes aniónicos, surfactantes catiónicos, surfactantes no-iónicos, y modificadores biológicos

de superficie activa), a ser combinado con el primer modificador de superficie (fosfolípido conjugado con un polímero hidrofílico o soluble en agua), con el fin de estabilizar las partículas formadas frente a la agregación de las mismas.

A este respecto, se traen a colación los ejemplos 19 a 21, los cuales exponen el uso específico de N-metil-2-pirrolidinona (NMP) como primer solvente miscible en agua, una solución acuosa al 2% (p/v) de glicerina como segundo solvente acuoso, mPEG-DSPE como primer modificador de superficie y Poloxámero 188 como segundo modificador de superficie, en donde el ingrediente activo se incorpora a una concentración de 1% (p/v), y la glicerina corresponde al excipiente usado durante la preparación farmacéutica. En este sentido, se debe entender que los compuestos y la concentración mencionada corresponden a modalidades preferentes y muestras de las opciones contempladas en las páginas 17 a 20, de acuerdo a las definiciones dadas a los términos "primer solvente", "segundo solvente", "modificador de superficie", "fosfolípido", "polímero hidrofílico", "surfactantes aniónicos", "surfactantes catiónicos", "surfactantes no-iónicos" y "modificadores biológicos de superficie activa".

Con base en lo anterior, se considera que la reivindicación 1 es concisa, por cuanto la descripción permite la interpretación de los compuestos usados en el método reclamado, así como las concentraciones de los ingredientes activos.

Así mismo, se desea poner de presente que la expresión "homogenizar la suspensión para formar una suspensión de pequeñas partículas" es concisa, toda vez que la descripción claramente enseña el uso de un homogenizador de pistón-abertura, como el homogenizador EmulsiFlex-C160 de Avestin Inc. (favor referirse a la página 21, líneas 25 a 28). Además, el ejemplo 19 enseña el mecanismo de homogenización de una pre-suspensión que comprende partículas de paclitaxel para formar una suspensión de pequeñas partículas precipitadas.

Igualmente, se desea aclarar a su Despacho que contrario a lo afirmado en el Oficio en cuestión, los ejemplos incluidos en la presente solicitud si tratan el método para preparar la composición farmacéutica de partículas submicrónicas de paclitaxel con las etapas definidas en la reivindicación. Como sustento de lo anterior, se llama la atención del Señor Examinador a los ejemplos de proceso A, B y C del Ejemplo 19, los cuales revelan claramente las etapas de:

(i) mezclar en el primer solvente miscible en agua o en el segundo solvente o en ambos primer solvente miscible en agua y segundo solvente, un primer modificador de superficie que incluye un fosfolípido conjugado con un polímero hidrofílico o soluble en agua (usando MPEG-DSPE);

(ii) mezclar en el primer solvente miscible en agua o en el segundo solvente o en ambos primer solvente miscible en agua y segundo solvente, un segundo modificador de superficie seleccionado del grupo consistente de: surfactantes aniónicos, surfactantes catiónicos, surfactantes no-iónicos, y modificadores biológicos de superficie activa (usando Poloxámero 188);

(iii) disolver el paclitaxel o sus compuestos derivados en el primer solvente miscible en agua (correspondiente al NMP) para formar una solución;

(iv) mezclar la solución con el segundo solvente para obtener una presuspensión de partículas; y

(v) homogenizar la presuspensión para formar una suspensión de pequeñas partículas precipitadas que tienen un tamaño de partícula efectivo promedio de menos de 1000 nm (específicamente un tamaño medio de partícula de 186 nm, 204 nm o 158 nm, respectivamente), como se reclama en el pliego reivindicatorio adjunto al presente memorial. Por lo tanto, se puede concluir que si existe sustento practico del método reclamado.

Por último, por favor nótese que las anteriores reivindicaciones 29 y 30 no han sido incluidas en el nuevo pliego reivindicatorio. Sin embargo, el solicitante no renuncia a la protección que pueda obtener sobre la misma, y se reserva el derecho de presentar una solicitud divisional que la contenga durante el trámite de la presente solicitud, beneficiándose de la fecha de prioridad inicialmente reivindicada

De esta manera, se considera que el pliego reivindicatorio define de manera clara y concisa la materia reclamada de acuerdo a las disposiciones del artículo 30. Así mismo, se puede concluir que la materia presentada en la solicitud tiene sustento suficiente en el capítulo descriptivo.

5.2. Argumentos a Favor del Nivel Inventivo de la Solicitud

En concordancia con el Artículo 18 de la Decisión 486, una invención se considerará inventiva si la misma no hubiese resultado obvia a partir del arte previo, para una persona del oficio normalmente versada en la materia. Esto a su vez implica que el Examinador a cargo de esta evaluación debe colocarse en el momento exacto en el que el inventor buscaba resolver el problema técnico contemplado en la solicitud y para el cual poseía la literatura y el conocimiento disponibles en dicho momento.

Siguiendo entonces los lineamientos de la Jurisprudencia aplicable al análisis del nivel inventivo de una solicitud de patente, se considera que una anterioridad o una combinación de dos anterioridades puede afectar dicho requerimiento de patentabilidad, si una persona medianamente versada en la materia encuentra enseñanzas o sugerencias claras que le indiquen cómo llegar a la invención reivindicada.

Ahora bien, el Examinador considera que los documentos D1 y D2 corresponden a los documentos del estado del arte más cercano a la presente solicitud, puesto que el documento D1 describe un método para preparar partículas compuestas por un núcleo poco hidrofílico, mientras que D2 enseña un procedimiento para preparar partículas de tamaño submicrónico de un compuesto orgánico donde la solubilidad del mismo es mayor en un primer solvente miscible en agua que en un

segundo solvente acuoso. Con base en lo anterior, se concluye que proveer un método para preparar una composición farmacéutica de partículas submicrónicas de paclitaxel o de sus compuesto derivados resulta evidente para el técnico medio en la materia, partiendo del método para preparar partículas de un compuesto orgánico de baja solubilidad en agua de acuerdo a D1 en el procedimiento de D2.

En este sentido, mi representada se encuentra en desacuerdo con el análisis llevado a cabo, por las siguientes razones:

La presente solicitud enseña un método para preparar una composición farmacéutica de partículas submicrónicas de paclitaxel precipitado o de sus compuestos derivados, la solubilidad de la cual es mayor en un primer solvente miscible en agua que en un segundo solvente que es acuoso, el método incluye los pasos de:

(i) mezclar en el primer solvente miscible en agua o en el segundo solvente o en ambos primer solvente miscible en agua y segundo solvente, un primer modificador de superficie que incluye un fosfolípido conjugado con un polímero hidrofílico o soluble en agua;

(ii) mezclar en el primer solvente miscible en agua o en el segundo solvente o en ambos primer solvente miscible en agua y segundo solvente, un segundo modificador de superficie seleccionado del grupo consistente de: surfactantes aniónicos, surfactantes catiónicos, surfactantes no-iónicos, y modificadores biológicos de superficie activa;

(iii) disolver el paclitaxel o sus compuestos derivados en el primer solvente miscible en agua para formar una solución;

(iv) mezclar la solución con el segundo solvente para obtener una presuspensión de partículas; y

(v) homogenizar la presuspensión para formar una suspensión de pequeñas partículas precipitadas que tienen un tamaño de partícula efectivo promedio de menos de 1000 nm.

Por el contrario, el documento D1 describe un método para preparar partículas de compuestos pobremente hidrofílicos/lipofílico, el cual comprende la etapa de disolver el compuesto pobremente hidrofílico/lipofílico en un solvente orgánico apropiado, seguido de una etapa de inyección lenta en una solución acuosa con el fin de formar las partículas del compuesto (véase D1, página 12, líneas 14 a 19). Sin embargo, D1 falla en enseñar o sugerir el uso de un primer modificador de superficie que comprende un fosfolípido conjugado con un polímero hidrofílico o soluble en agua, y un segundo modificador de la superficie seleccionado de surfactantes aniónicos, catiónicos, no-iónicos y modificadores biológicos de superficie activa en combinación con una etapa de homogeneización, de acuerdo a la invención bajo estudio.

Por su parte, D2 divulga un procedimiento para preparar una suspensión de un compuesto farmacéuticamente activo, como es indicado en la página 1, líneas 9 a 11 de dicho documento. No obstante, no existe indicio o sugerencia alguna en el texto que enseñe cómo preparar específicamente una composición farmacéutica de partículas submicrónicas de paclitaxel o sus compuestos derivados. Adicionalmente, el documento D2 falla en enseñar el uso de modificadores de superficie que comprenden un fosfolípido conjugado con un polímero hidrofílico o soluble en agua. Así, D2 no define o sugiere la combinación específica de modificadores de superficie reclamada en la reivindicación 1, y mucho menos que estos sean incorporados para posteriormente ser sometidos a una etapa de homogenización, como se indica en el pliego reivindicatorio.

En vista de lo anterior, resulta claro que una persona medianamente versada en la materia no tendría motivación alguna en combinar las enseñanzas de D2 y D1 para así alcanzar la invención definida en la presente solicitud, por cuanto dichos documentos tiene objetivos completamente diferentes. Como sustento de lo anterior, se desea poner de presente que las combinaciones de agentes surfactantes descritos en D1 se utilizan para proporcionar un vehículo para solubilizar los compuestos pobremente hidrofílicos/lipofílicos, con el fin de promover la entrega del mismo (véase D1 en la página 1, línea 34 a la página 2, línea 1). Por el contrario, las formulaciones descritas en D2 son formulaciones estables de partículas en las que el agente activo no se encuentra solubilizado. Así las cosas, un experto en la materia no se vería versado a incluir la combinación de surfactantes de acuerdo a D1 en el proceso de D2, toda vez que buscar la solubilidad del ingrediente activo va en contra de las bases y objetivos de dicho proceso.

Lo que es más, al no describe o sugerir el uso del primer y segundo modificador de superficie mencionados en la reivindicación 1, para posteriormente ser sometidos a una etapa de homogenización, no se logra la producción de formulaciones estables de partículas de paclitaxel, las cuales no generan una agregación significativamente entre ellas bajo las condiciones de las pruebas de estrés. La estabilidad arriba mencionada se logra al aplicar el método de preparación de una composición farmacéutica de partículas submicrómicas de paclitaxel precipitado o sus compuestos derivados, como se demuestra en el ejemplo A del ejemplo 19, en el ejemplo 21, y en las figuras 19 y 20 de la presente solicitud.

Adicionalmente, se desea presentar ante su Despacho una copia del documento US 2006/0073199, en el cual se presentan datos comparativos entre las composiciones farmacéuticas producidas de acuerdo al método de la presente invención, frente a una formulación similar que sólo contiene paclitaxel y MPEG-DSPE 2000. Específicamente, el documento US 2006/0073199 demuestra que cuando ambos DSPE-MPEG 2000 y Poloxamer 188 (primer y segundo modificador de superficie) se incluyeron en una formulación de nanopartículas de paclitaxel preparados de acuerdo con el método reivindicado, la composición no presento un aumento significativo en el tamaño de partícula, o agregación después de las pruebas de estrés, lo cual corresponde a un resultado de manera

inesperado y ventajoso (véase el ejemplo 3 y la figura 4 de US 2006/0073199). En contraste, cuando sólo se incluyó DSPE-MPEG 2000 en la formulación de nanopartículas de paclitaxel manteniendo el resto de condiciones constantes de acuerdo al método reclamado, la composición mostró un aumento del tamaño de partícula y aumento de la agregación después de la prueba de estrés (véase el Ejemplo 3 y la Figura 6 de US 2006/0073199). Así las cosas, los resultados presentados en el documento adjunto al presente memorial demuestran que las composiciones preparadas con la combinación específica de surfactantes presenta de manera inesperada y ventajosa una disminución en la agregación y en el aumento del tamaño de partícula después de pruebas de estrés en comparación con una composición preparada con sólo uno de los surfactantes, lo que permite obtener composiciones farmacéuticas de paclitaxel más estables.

De acuerdo a lo anterior, una persona medianamente versada en la materia no tendría motivación alguna en combinar las enseñanzas de las formulaciones expuestas en los documentos D1 y D2 para alcanzar así el método de preparación de las composiciones farmacéuticas de partículas sumbicrónicas de paclitaxel precipitado de acuerdo a la presente solicitud, puesto que no existe indicio alguno en los documentos citados que lleve a una experto a incluir los modificadores de superficies, para después someter la presuspensión a una etapa de homogenización según lo reclamado en la presente solicitud. De la misma manera, no existe sugerencia alguna que lleve a un experto en la materia a alcanzar los resultados sorprendentes relacionados con la estabilidad obtenida al no presentarse un incremento del tamaño de partícula o de agregación de las mismas.

En vista de lo anterior, se considera que D1 y D2 tomados por separados o en combinación resultan insuficientes para que la persona medianamente versada en la materia pueda deducir de manera obvia todas las características de la preparación sólida de acuerdo a la presente invención, ya que dichas anterioridades no dan indicios suficientes sobre la forma cómo se solucionó el problema técnico. Dado lo anterior, consideramos que las anterioridades citadas por el Examinador no pueden ser consideradas como anterioridades válidas capaces de afectar el nivel inventivo

6. Conclusiones

Finalmente, puesto que se han atendido a cabalidad las observaciones hechas por su Despacho sobre la presente solicitud de patente, respetuosamente solicito se continúe con su tramitación. Sin embargo, en el evento que el Despacho considere que una explicación adicional es necesaria, respetuosamente le solicito hacer uso de la facultad que le otorga el artículo 45 inciso 2 de la Decisión 486 de la Comisión de la Comunidad Andina a efecto de requerir nuevamente de nuestra parte cualquier complementación o aclaración.

7. Anexos

- Copia autenticada de la sustitución del poder otorgado por la Sociedad Solicitante que hizo el Doctor EMILIO FERRERO a mi favor, y para lo cual solicito me sea reconocida personería para continuar con el trámite del asunto de la referencia.
- Nuevo pliego reivindicatorio, conformado por 30 reivindicaciones.
- Comprobante de pago por valor de $ 600.000 por veinte (20) reivindicaciones adicionales después de la décima reivindicación.
- Copia del documento US 2006/0073199.
- Formulario para modificaciones.

Señor Director,

LUZ CLEMENCIA DE PÁEZ
C.C. No. 35.456.344 de Usaquén
T.P.A. No. 23.555

COLO-05873-841-020-6
COLO-05873-264-001-4
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No. M-2012-212676\LLG

Industria y Comercio
SUPERINTENDENCIA

Espacio reservado para el adhesivo de radicación

FORMULARIO UNICO DE CORRECCIONES Y MODIFICACIONES

1 SOLICITUD DE:

☐ Corrección de Error Material ☒ Modificación a una solicitud

2 SOLICITANTE

Nombre: BAXTER INTERNATIONAL INC
Sociedad constituida y existente bajo las leyes del Estado de Delaware, Estados Unidos de América

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Deerfield 60015
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IDENTIFICACION

C.C. ☐ NIT ☐
C.E. ☐ Otro ☐

Número: _____

3 REPRESENTANTE O APODERADO

Nombre: Luz Clemencia de PAEZ
Dirección: Carrera 4ª No. 72-35
Teléfono: 347 36 11 **Fax:** 217 92 11
E-mail: cavelier@cavelier.com

IDENTIFICACION

C.C. ☒ NIT ☐
C.E. ☐ Otro ☐

Número: 35.456.344 TP 23.555

4 Número de expediente: 06.042.082

5 Descripción de la corrección o modificación solicitada:

Presento nuevo capítulo reivindicatorio compuesto por 30 reivindicaciones con el fin de superar las objeciones hechas por Su Despacho a la presente solicitud de patente y pido respetuosamente que el examen de patentabilidad sea realizado sobre estas nuevas reivindicaciones presentadas.

6

Comprobante de pago No. 12-0022696 **Fecha:** 05 de marzo de 2012
Comprobante de pago No. 12-0024911 **Fecha:** 12 de marzo de 2012

7 Anexos

☐ Comprobante de pago de la tasa por $120.000.oo, por concepto de modificaciones.

☒ Comprobante de pago por 20 reivindicaciones adicionales después de la décima reivindicación por $600.000.oo

☐ Poderes, si fuere el caso

☐ Documento que acredita la existencia y representación legal de las partes, cuando sean personas jurídicas.

☒ Copia auténtica de la sustitución que del poder otorgado por la Sociedad Solicitante hizo el Doctor EMILIO FERRERO a mi favor, y para lo cual solicito me sea reconocida personería para continuar con el trámite del asunto de la referencia.

8 **NOMBRE:** LUZ CLEMENCIA DE PAEZ **FIRMA:** Luz Clemencia de Páez

C.C. 35.456.344 de Usaquén **T.P.A.** 23.555

LLG

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Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

E. S. D.

Poderdante : **BAXTER INTERNATIONAL INC.**

Poder conferido el : 16/06/1993

Yo, EMILIO FERRERO, obrando como apoderado principal del poderdante arriba mencionado, mediante el presente escrito sustituyo el poder a mi otorgado a la doctora LUZ CLEMENCIA DE PAEZ, identificada con cédula de ciudadanía No. 35.456.344 y Tarjeta Profesional N°23.555, con todas las facultades que me fueron conferidas.

Hago la anterior sustitución conforme al artículo 66 del Código de Procedimiento Civil.

Señora Jefe,

EMILIO FERRERO
T.P.A. N° 31.929
C.C. N° 438.019 de Usaquén

Acepto:

LUZ CLEMENCIA DE PAEZ
T.P.A. N° 23.555
C.C. No. 35.456.344 de Usaquén

NOTARIA SEXTA DE BOGOTA
RECONOCIMIENTO Y PRESENTACION PERSONAL
Bogotá, D.C. 22 OCT 2009
Ante mi AMPARO QUINTERO ARTURO,
NOTARIA SEXTA DEL CIRCULO DE
BOGOTA, D.C.
Compareció(eron)
Quien(es) exhibió(eron) la(s) C.C.
Y declaró(aron) que la(s) firma(s) que aparece(n)
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En constancia se firma esta diligencia.

REPÚBLICA DE COLOMBIA
Amparo Quintero Arturo
NOTARIA
NOTARIA SEXTA DE BOGOTA

COMO NOTARIA SEXTA DEL CIRCULO DE BOGOTÁ, D.C. HAGO CONSTAR QUE LA FOTOCOPIA COINCIDE CON EL ORIGINAL QUE HE TENIDO A LA VISTA.
02 MAR 2012
AMPARO QUINTERO ARTURO
NOTARIA

REIVINDICACIONES

1. Un método para preparar una composición farmacéutica de partículas submicrónicas de paclitaxel precipitado o de sus compuestos derivados, la solubilidad de la cual es mayor en un primer solvente miscible en agua que en un segundo solvente que es acuoso, el método incluye los pasos de:

(i) mezclar en el primer solvente miscible en agua o en el segundo solvente o en ambos primer solvente miscible en agua y segundo solvente, un primer modificador de superficie que incluye un fosfolípido conjugado con un polímero hidrofílico o soluble en agua;

(ii) mezclar en el primer solvente miscible en agua o en el segundo solvente o en ambos primer solvente miscible en agua y segundo solvente, un segundo modificador de superficie seleccionado del grupo consistente de: surfactantes aniónicos, surfactantes catiónicos, surfactantes no-iónicos, y modificadores biológicos de superficie activa;

(iii) disolver el paclitaxel o sus compuestos derivados en el primer solvente miscible en agua para formar una solución;

(iv) mezclar la solución con el segundo solvente para obtener una presuspensión de partículas; y

(v) homogenizar la presuspensión para formar una suspensión de pequeñas partículas precipitadas que tienen un tamaño de partícula efectivo promedio de menos de 1000 nm.

2. El método de la reivindicación 1, en donde el fosfolípido es natural o sintético.

3. El método de la reivindicación 1, en donde el fosfolípido es fosfatidilcolina, fosfatidiletanolamina, diacil-glicero-fosfoetanolamina, fosfatidilserina, fosfatidilinositol, fosfatidilglicerol, ácido fosfatídico, lisofosfolípidos, fosfolípidos de huevo o soya o una combinación de ellos.

4. El método de la reivindicación 3, en donde el diacil-glicero-fosfoetanolamina es seleccionado de un grupo consistente de dimiristoil-glicero-fosfoetanolamina (DMPE), dipalmitoil-glicero-fosfoetanolamina (DPPE), distearoil-glicero-fosfoetanolamina (DSPE), dioleolil-glicero-fosfoetanolamina (DOPE).

5. El método de la reivindicación 1, en donde el polímero soluble o hidrofílico que se conjuga con el fosfolípido es polietileno glicol (PEG).

6. El método de la reivindicación 5, en donde el PEG es seleccionado del grupo que consistente de PEG 350, PEG 550, PEG 750, PEG 1000, PEG 2000, PEG-3000 y PEG 5000.

7. El método de la reivindicación 1, en donde el polímero soluble o hidrofílico que se conjuga con el fosfolípido es seleccionado de un grupo consistente de: dextran, hidroxipropil metacrilato (HPMA) y poliglutamato.

8. El método de la reivindicación 1, en donde el segundo modificador de superficie es un copolímero de oxietileno y oxipropileno.

9. El método de la reivindicación 8, en donde el copolímero de oxietileno y oxipropileno es un copolímero en bloque.

10. El método de la reivindicación 1, en donde el segundo modificador de superficie es poloxamer.

11. El método de la reivindicación 1, en donde el primer solvente miscible en agua es N-metil-2-pirrolidinona.

12. El método de la reivindicación 1, en donde la homogenización es realizada cerca de los 30°C o más.

13. El método de la reivindicación 1, en donde las pequeñas partículas tienen un tamaño de partícula efectivo promedio de menos de 400 nm.

14. El método de la reivindicación 1, en donde las pequeñas partículas tienen un tamaño de partícula efectivo promedio de menos de 200 nm.

15. El método de la reivindicación 1, en donde las pequeñas partículas tienen un tamaño de partícula efectivo promedio de menos de 150 nm.

16. El método de la reivindicación 1, que incluye además la esterilización de la composición.

17. El método de la reivindicación 16, en donde la esterilización de la composición incluye filtrado estéril de la solución y del segundo solvente antes de mezclar y realizar los pasos subsiguientes bajo condiciones de asepsia.

18. El método de la reivindicación 16, en donde la esterilización de la composición incluye filtrado estéril de las partículas.

19. El método de la reivindicación 16, en donde la esterilización comprende esterilización por calor.

20. El método de la reivindicación 19, en donde la esterilización por calor es efectuada por calor dentro del homogenizador en el cual el homogenizador sirve como una fuente de calentamiento y presurización para la esterilización.

21. El método de la reivindicación 16, en donde la esterilización incluye radiaciones gamma.

22. El método de la reivindicación 1, que incluye además la remoción del primer solvente miscible en agua de la suspensión.

23. El método de la reivindicación 22, en donde la remoción del primer solvente miscible en agua es por filtración.

24. El método de la reivindicación 23, en donde la filtración es ultrafiltración de flujo cruzado.

25. El método de la reivindicación 22, en donde la remoción del primer solvente miscible en agua es simultánea con la homogenización.

26. El método de la reivindicación 1, que incluye además la remoción de la fase líquida de la suspensión para formar un polvo seco de partículas, en donde la remoción de la fase líquida es seleccionada de un grupo que consiste de: evaporación, evaporación rotativa, liofilización, secado-congelado, diafiltración, centrifugación, fraccionamiento de campo de fuerza, filtración de alta presión y osmosis reversa.

27. El método de la reivindicación 26, que incluye además la adición de un diluyente al polvo seco.

28. El método de la reivindicación 1, en donde las partículas no son solubles.

29. El método de la reivindicación 1, en donde las partículas no se agregan bajo condiciones de esfuerzo o almacenamiento.

30. Una composición farmacéutica de partículas submicrónicas de paclitaxel precipitado o docetaxel, la cual comprende una suspensión de pequeñas partículas precipitadas de un ingrediente activo, un fosfolípido conjugado con un polímero hidrofílico o soluble en agua, y un copolímero de oxietileno y oxipropileno es un copolímero en bloque, las pequeñas partículas precipitadas tienen un tamaño de partícula efectivo promedio de menos de 1000 nm, y el ingrediente activo corresponde a paclitaxel o docetaxel.

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Chaubal et al. (43) **Pub. Date: Apr. 6, 2006**

(54) **SURFACTANT SYSTEMS FOR DELIVERY OF ORGANIC COMPOUNDS**

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(21) Appl. No.: **11/224,633**

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Related U.S. Application Data

(63) Continuation-in-part of application No. 10/703,395, filed on Nov. 7, 2003, which is a continuation-in-part of application No. 10/390,333, filed on Mar. 17, 2003, which is a continuation-in-part of application No. 10/246,802, filed on Sep. 17, 2002, which is a continuation-in-part of application No. 10/035,821, filed on Oct. 19, 2001, now Pat. No. 6,977,085, which is a continuation-in-part of application No. 09/953,979, filed on Sep. 17, 2001, now Pat. No. 6,951,656, which is a continuation-in-part of application No. 09/874,637, filed on Jun. 5, 2001, now Pat. No. 6,869,617.

(60) Provisional application No. 60/258,160, filed on Dec. 22, 2000.

Publication Classification

(51) **Int. Cl.**
A61K 9/127 (2006.01)

(52) **U.S. Cl.** **424/450**

(57) **ABSTRACT**

Submicron particles of an organic compound, such as therapeutic and diagnostic agent are disclosed. The organic compound particles are associated with at least two surfactants including a block copolymer and phospholipids conjugated with a hydrophilic polymer.

FIG. 1: Method A

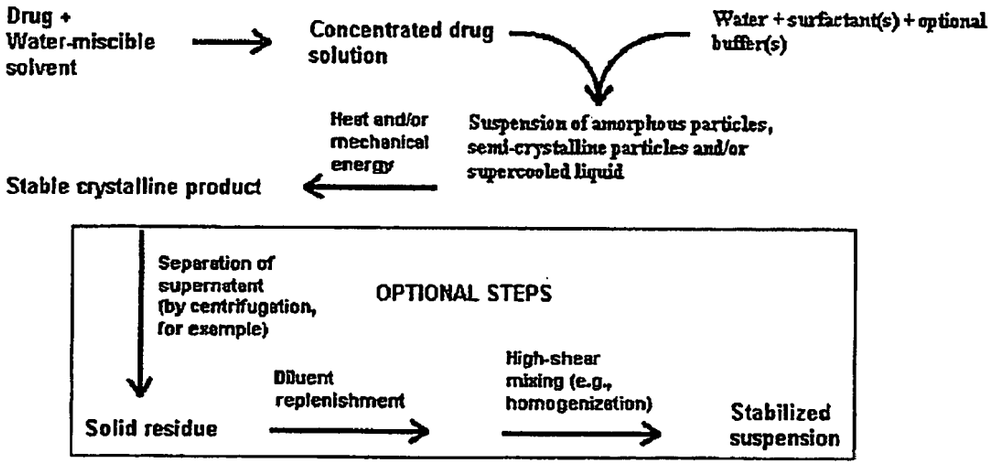


FIG. 2: Method B

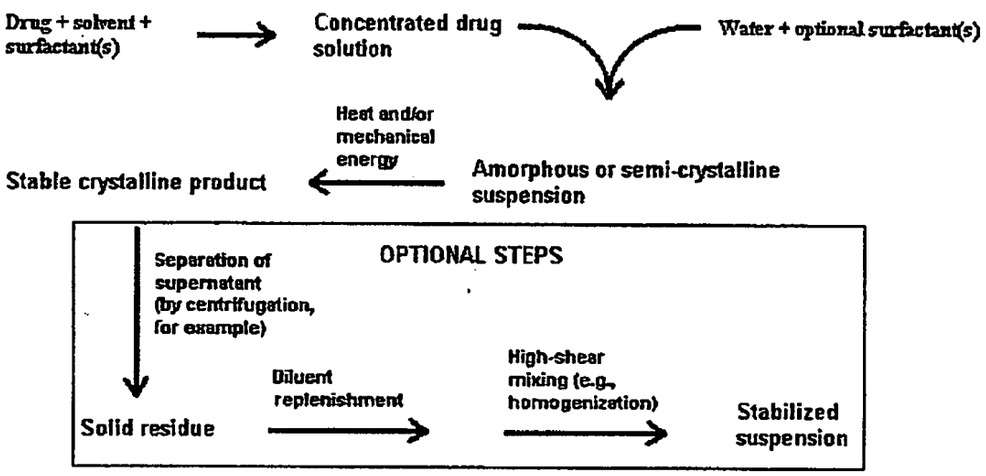


FIG. 3 · Dissolution of two formulations of submicron paclitaxel

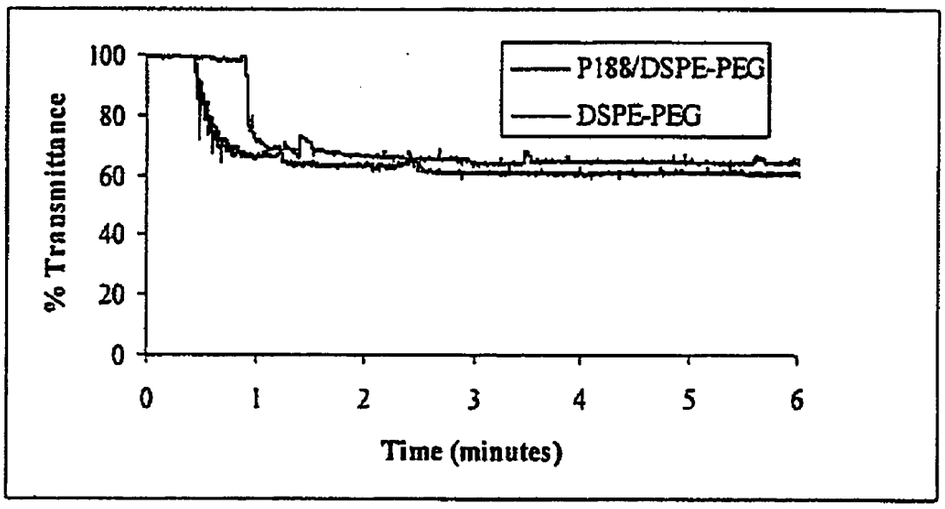


FIG. 4 Effect of various stressed conditions on the particle size of submicron particles of paclitaxel

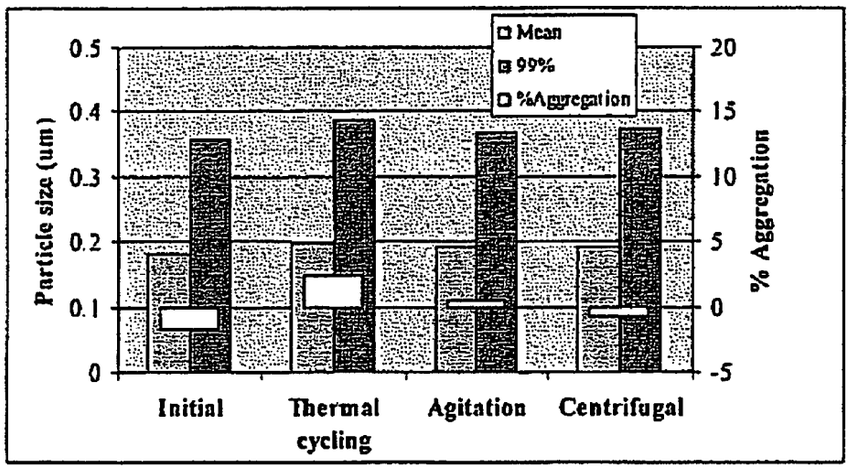


FIG. 5: Effect of storage on the particle size of submicron particles of paclitaxel

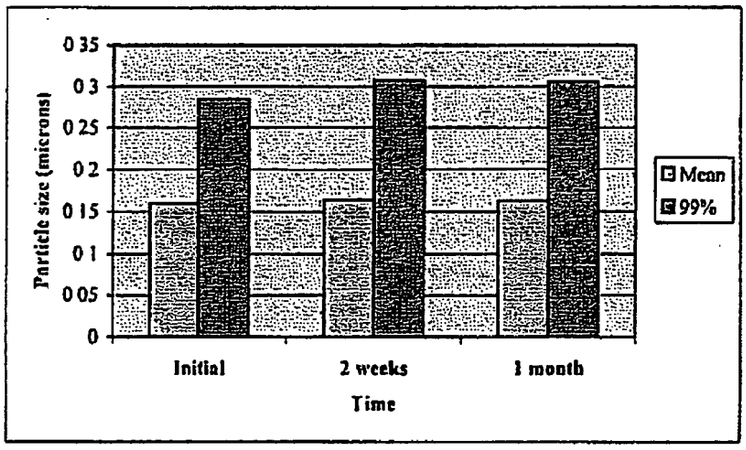


FIG. 6

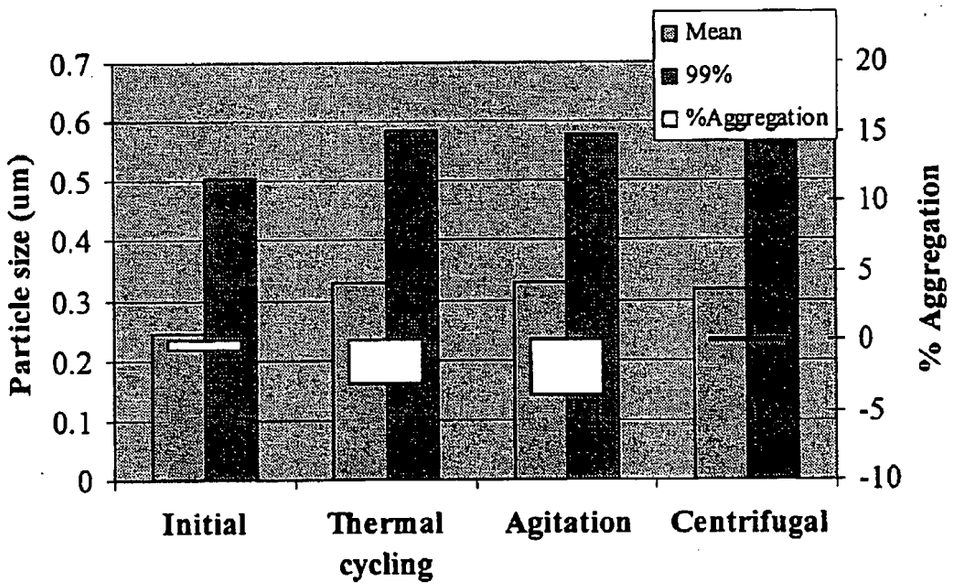

Mean
99%
%Aggregation
Particle size (um)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
20
15
10
5
0
-5
-10
% Aggregation
Initial
Thermal cycling
Agitation
Centrifugal

FIG. 7: Effect of stress on particle size

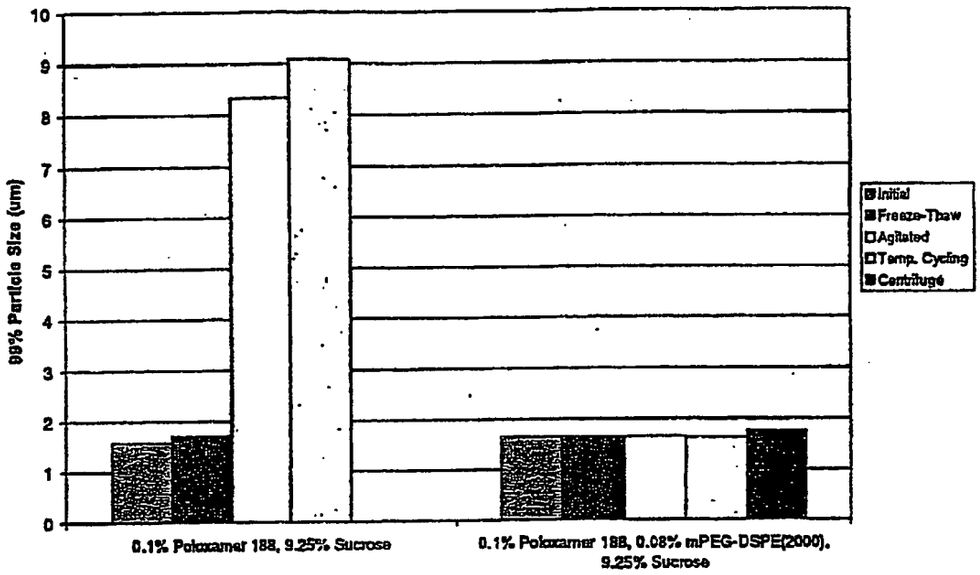
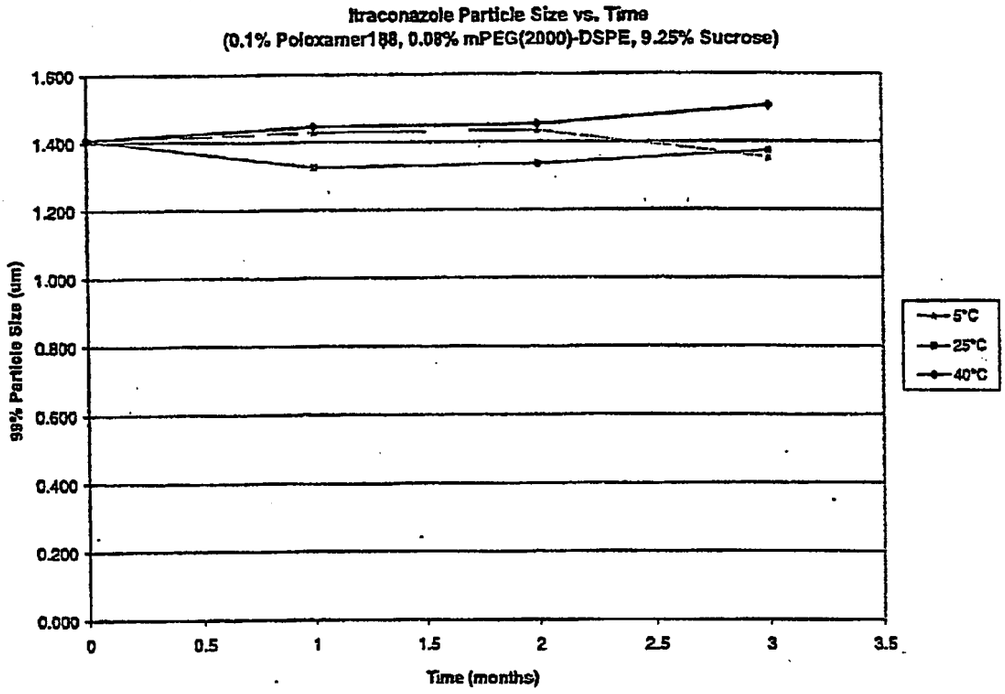


FIG. 8: Effect of storage on particle size



SURFACTANT SYSTEMS FOR DELIVERY OF ORGANIC COMPOUNDS

CROSS-REFERENCE TO RELATED APPLICATIONS:

[0001] This application is a continuation-in-part of application Ser. No. 10/703,395, filed on Nov. 7, 2003 which is a continuation-in-part of application Ser. No. 10/390,333 filed on Mar. 17, 2003, which is a continuation-in-part of application Ser. No. 10/246,802 filed on Sep. 17, 2002, which is a continuation-in-part of application Ser. No. 10/035,821 filed on Oct. 19, 2001, which is a continuation-in-part of application Ser. No. 09/953,979 filed Sep. 17, 2001 which is a continuation-in-part of application Ser. No. 09/874,637 filed on Jun. 5, 2001, which claims priority from provisional application Ser. No. 60/258,160 filed Dec. 22, 2000. All of the above-referenced applications are incorporated herein by reference and made a part hereof.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not Applicable.

BACKGROUND OF THE INVENTION

[0003] 1. Technical Field

[0004] The present invention relates to systems and methods for the delivery of insoluble or poorly soluble organic compound, such as therapeutic, and/or diagnostic agents. More particularly, the present invention relates to surfactants and surfactant systems used with such compounds.

[0005] 2. Background Art

[0006] Many organic compounds that are useful in therapeutic or diagnostic applications are poorly soluble or insoluble in aqueous environments. Because of their poor solubility such compounds present challenges to delivering them by traditional administrative routes. Recently, efforts have been made to develop methods and systems whereby such non-soluble and poorly soluble compounds can be delivered to a patient. These efforts have led to the development of different types of drug delivery vehicles.

[0007] One approach for delivering poorly soluble or insoluble compounds is to formulate the compound as a solid particle suspension. Compounds that are insoluble in water can be formulated as a stable suspension of submicron sized particles in an aqueous medium such as microparticulate or nanoparticulate suspensions. In this way, compounds that were previously unable to be formulated in an aqueous based system can be made suitable for intravenous administration.

[0008] Solid particle suspensions of nanoparticles are commonly referred to as nanosuspensions. Nanoparticles range in particle size from about 10 nm to about 10 microns, preferably from about 100 nm to about 2 microns and, more preferably, from about 400 nm to about 1,000 nm. Nanoparticles also are generally coated with or have associated on their surfaces one or more surfactants or other excipients in order to prevent agglomeration, flocculation or what are referred to as Ostwald ripening of the nanoparticles.

[0009] Sub-micron sized particles generally and nanoparticles specifically may be administered parenterally. In addition, preparations of small particles of water insoluble compounds may also be suitable for oral, pulmonary, topical, ophthalmic, nasal, buccal, rectal, vaginal, transdermal, or other routes of administration. The small size of the particles improves the dissolution rate of the compound, and hence improves its bioavailability and potentially its toxicity profiles. The particle size will depend on the route of administration, formulation, solubility, and bioavailability of the compound. For example, for oral administration, it is desirable to have a particle size of less than about 7 microns. For pulmonary administration, the particles are preferably less than about 10 microns in size.

[0010] One approach for preparing a nanosuspension is described in U.S. Pat. No. 6,607,784, assigned to the assignee of the present application and incorporated herein by reference and made a part hereof. The '784 patent discloses a method for preparing submicron sized particles of an organic compound, wherein the solubility of the organic compound is greater in a water-miscible selected solvent than in another solvent which is aqueous. The process described in the '784 patent generally includes the steps of (i) dissolving the organic compound in the water-miscible selected solvent to form a first solution, (ii) mixing the first solution with a second solvent to precipitate the compound to define a pre-suspension; and (iii) adding energy to the pre-suspension to form particles which can be of submicron size. Often, the average effective particle size can range between about 400 nm to 1,000 nm or below, extending into low micron size, typically no greater than about 2 microns.

[0011] Other examples of insoluble compound delivery systems and methods are disclosed in U.S. Pat. Nos. 5,858,410 and 5,922,355. The '355 patent discloses providing submicron sized particles of insoluble compounds 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.

[0012] Another approach for providing formulations of insoluble compounds for parenteral delivery is disclosed in U.S. Pat. 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 particle having an average effective particle size of less than 400 nm. The surface modifier is adsorbed on the surface of the particle in an amount sufficient to prevent agglomeration into larger particles.

[0013] In addition to nanoparticulates and nanosuspensions discussed above, other vehicles for delivering insoluble or poorly soluble compounds have also been considered. These include micelles, liposomes, microemulsions, emulsions, nanocapsules, and other dispersed phase (including nanodispersed phase) systems.

[0014] Micelles have been considered as vehicles for delivering pharmaceutical components. Micelles are typically spherical structures comprised of a conglomeration of surfactant molecules, formed as a result of the interaction between the hydrophobic parts of the surfactant molecules.

[0015] Liposomes have also been considered as vehicles for delivering pharmaceutical components. Liposomes are comprised of a conglomeration of surfactant molecules having one or several bi-layer structures, normally compris-

ing lipid with an aqueous cavity therein. Liposomes possess the capability to incorporate both water-soluble and oil-soluble substances.

[0016] Organic compounds such as therapeutic or diagnostic agents may also be delivered in a microemulsion or an emulsion. Microemulsions are bicontinuous structures having only a monolayer wall, comprised generally of water, oil and surfactant(s), (which constitute a single optically isotropic and thermodynamically stable liquid solution.) The size of microemulsion droplets ranges from about 10-100 nm. Microemulsions have the capacity to solubilize both water-soluble and oil-soluble compounds. Accordingly, for delivery, microemulsions can be comprised of oil droplets in an aqueous continuum, water in an oil continuum, or a bicontinuous structures. Other suitable vehicles include cubosomes, which are dispersions of one of the bicontinuous cubic phases that have a lamellar wall, and hexasomes which are dispersions of hexagonal phases that have a lamellar wall.

[0017] As noted above, other vehicles for delivering organic compounds such as therapeutic or diagnostic agents are emulsions. Emulsions comprise droplets which are relatively large in size (as compared to microemulsions.) In contrast to microemulsions which form spontaneously, emulsions must be prepared with the input of energy. Formation of emulsions includes high pressure homogenization for producing emulsion droplets (ranging in size from about 100 nm-10 μm) and generating a new surface thereon. Emulsions may be water-in-oil or oil-in-water based on surfactants, oil and water volume fraction, temperature, salt concentration, and the presence of co-surfactants and other co-solutes.

[0018] One challenge in the area of insoluble organic compound delivery is that many of the poorly soluble or insoluble compounds, when formulated into submicron sized particles such as nanoparticles, and the like, exhibit undesirable particle growth and/or aggregation (e.g., agglomeration), due in part to the surface activity of the compound. Accordingly, the particle, (emulsion or suspension) may include a selected amount of one or more surfactants that protect the particle form such growth and aggregation, and generally stabilize the particle or other composition and the active ingredient therein.

[0019] Surfactants are generally low to moderate weight compounds which contain a hydrophobic portion, which is generally readily soluble in oil, but sparingly soluble or insoluble in water, and a hydrophilic portion, which is sparingly soluble or insoluble in oil, but readily soluble in water. In addition to protecting against growth and aggregation and stabilizing the organic compound delivery vehicle, surfactants are also useful as excipients in organic compound delivery systems and formulations because they increase the effective solubility of an otherwise poorly soluble or non-soluble organic compound, and may decrease hydrolytic degradation, decrease toxicity and generally improve bioavailability. They may also provide selected and advantageous effects on drug release rate and selectivity of drug uptake. Surfactants are generally classified as either anionic, cationic, or nonionic.

[0020] Some surfactants suffer from poor physiological compatibility upon injection and/or do not provide effective long term stability. In addition, sub-micron sized particles and other organic compound delivery vehicles that are injected intravenously are often recognized and scavenged by the reticoendothelial system (RES) of the liver, thereby preventing the nanoparticles from being delivered to the target organs.

[0021] Thus, it would be desirable to provide a surfactant or combination of surfactants that are effective stabilizers of the submicron sized particles or other organic compound delivery vehicle or system, are safe and physiologically compatible, and protect the particle or other vehicle from being scavenged by the RES of the liver.

SUMMARY OF THE INVENTION

[0022] In one aspect, the present invention is directed to a composition including an organic compound and at least two surfactants associated therewith. The surfactants include a block copolymer of polyoxyethylene and polyoxypropylene combined with an amphiphilic compound conjugated with a hydrophilic polymer.

[0023] In another aspect, the present invention is directed to a composition including an organic compound and at least two surfactants associated therewith. Relative to each other, the at least two surfactants include approximately 90-10% (w/v) of the block copolymer $HO(CH_2CH_2O)_{80}(CH_3H_6O)_{27}(CH_2CH_2O)_{80}H$ and approximately 10-90% (w/w) of an amphiphilic compound conjugated with a hydrophilic polymer.

[0024] In another aspect, the present invention is directed to a composition including an organic compound and at least two surfactants associated therewith. Relative to each other, the at least two surfactants include approximately 90-10% (w/w) of a block copolymer of polyethylene and polyoxypropylene and approximately 10-90% (w/w) of a peglyated phosphatidyl ethanolamine.

[0025] In another aspect, the present invention is directed to a method for making submicron particles of an organic compound, the particles having a mean particle size of about 10 nm to about 10 μm. The method includes dissolving the compound in a water miscible solvent to form a first solution and preparing an aqueous solution including at least two surfactants. Relative to each other, the at least two surfactants include approximately 90-10% (w/w) of a block copolymer of polyoxyethylene and polyoxypropylene and approximately 10-90% (w/w) of an amphiphilic compound conjugated with a hydrophilic polymer. The method further includes adding the first solution to the aqueous solution to form a presuspension, and adding energy to the presuspension.

[0026] In still another aspect, the present invention is directed to a method for making particles of an organic compound, the particles having a mean particle size of about 10 nm to about 10 μm. The method includes adding an organic compound and at least two surfactants wherein, relative to each other, the at least two surfactants 90-10% (w/w) of a block copolymer of polyoxyethylene and polyoxypropylene and 10-90% (w/w) of a hydrophilic polymer conjugated with a phospholipid to a milling apparatus and forming a mixture. A suitable grinding media is also added to the milling apparatus. The method further includes milling the mixture in the presence of the grinding media.

[0027] In a further aspect, the present invention is directed to a method of administering an effective amount of a

composition comprising particles including an organic compound associated with at least two surfactants to a subject. The particles have a mean particle size of about 10 nm to about 10 μm. Relative to each other, the at least two surfactants include 90-10% (w/w) of a block copolymer of polyoxyethylene and polyoxypropylene and 10-90% (w/w) of an amphiphilic compound conjugated with a hydrophilic compound.

[0028] These and other aspects and attributes of the present invention will be discussed with reference to the following drawings and accompanying specification.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] **FIG. 1** is a diagrammatic representation of one method of making nanoparticles;

[0030] **FIG. 2** is a diagrammatic representation of another method of making nanoparticles;

[0031] **FIG. 3** shows the dissolution of two formulations of submicron (paclitaxel) particles with the mPEG-DSPE/Poloxamer surfactant system of the present invention;

[0032] **FIG. 4** shows the effect of various stressed conditions on the particle size of submicron (paclitaxel) particles with the mPEG-DSPE/Poloxamer surfactant system of the present invention;

[0033] **FIG. 5** shows the effect of storage on the particle size of submicron (paclitaxel) particles with the mPEG-DSPE/Poloxamer surfactant system of the present invention;

[0034] **FIG. 6** shows the effects of various stress conditions on the particle size of submicron (paclitaxel) particles with mPEG-DSPE surfactant only;

[0035] **FIG. 7** shows the effects of stress on the particle size of nanoparticles of the drug itraconazole with the surfactant system of the present invention and a poloxamer surfactant only; and

[0036] **FIG. 8** shows the long-term stability of nanoparticles made with the surfactant system of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0037] The present invention may be embodied in many different forms. Preferred embodiments of the invention are disclosed with the understanding that the present disclosure is to be considered as an exemplification of the principles of the invention and are not intended to limit the broad aspects of the invention to the embodiments illustrated.

[0038] The present invention provides compositions of non-soluble or poorly soluble organic compounds intended for delivery to a patient or human subject, typically for therapeutic or diagnostic purposes. 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, which can be selected from therapeutic agents, diagnostic agents, cosmetics, nutritional supplements, and pesticides. Examples of such agents and compounds are provided in U.S. patent application Ser. No. 10/703,395, filed Nov. 7, 2003, published as U.S. Publication No. 2004/0245662 A1, from which the present application claims priority and which is incorporated by reference in its entirety. In addition to therapeutic agents, diagnostic agents, such as x-ray imaging agents and contrast media can be prepared as submicron particles.

[0039] A description of classes of therapeutic agents and diagnostic agents and a listing of species within each class can also be found in Martindale, The Extra Pharmacopoeia, Twenty-ninth Edition, The Pharmaceutical Press, London, 1989 which is incorporated herein by reference and made a part hereof. The therapeutic agents and diagnostic agents are commercially available and/or can be prepared by techniques known in the art.

[0040] The agent or other organic compound is at least poorly water-soluble. What is meant by "poorly water soluble" is a solubility of the compound in water of less than about 10 mg/mL, and preferably less than 1 mg/mL. These poorly water-soluble agents are most suitable for aqueous suspension preparations since there are limited alternatives of formulating these agents in an aqueous medium.

[0041] In accordance with one preferred embodiment, the vehicle for delivering the compound may be in particulate form. Preferably, such particles have a mean particle size of generally less than about 100 μm 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). However, the particles can be prepared in a wide range of sizes, such as from about 20 μm to about 10 nm, from about 10 μm to about 10 nm, less than 2 μm and more particularly from about 2 μm to about 10 nm, from about 1 μm to about 10 nm, from about 400 nm to about 50 nm, from about 200 nm to about 50 nm or any range or combination of ranges therein. The preferred mean particle size depends on factors such as the intended route of administration, formulation, solubility, toxicity and bioavailability of the compound.

[0042] To be suitable for parenteral administration, the particles preferably have a mean particle size of less than about 7 μm, and more preferably less than about 2 μm or any range or combination of ranges therein. Parenteral administration includes intravenous, intra-arterial, intrathecal, intraperitoneal, intraocular, intra-articular, intradural, intraventricular, intrapericardial, intramuscular, intradermal or subcutaneous injection.

[0043] Particles sizes for oral dosage forms can be in excess of 2 μm. The particles can range in size up to about 100 μm, provided that the particles have sufficient bioavailability 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.

[0044] The present invention is further suitable for providing particles of the organic compound in a form suitable for pulmonary administration. Particles sizes for pulmonary dosage forms can be in excess of 500 nm and typically less than about 10 μm. The particles in the suspension can be aerosolized and administered by a nebulizer for pulmonary administration. Alternatively, the particles can be administered as dry powder by a dry powder inhaler after removing the liquid phase from the suspension, or the dry powder can be resuspended in a non-aqueous propellant for administration by a metered dose inhaler. An example of a suitable propellant is a hydrofluorocarbon (HFC) such as HFC-134a (1,1,1,2-tetrafluoroethane) and HFC-227ea (1,1,1,2,3,3,3-heptafluoropropane). Unlike chlorofluorcarbons (CFC's), HFC's exhibit little or no ozone depletion potential.

[0045] Dosage forms for other routes of delivery, such as nasal, topical, ophthalmic, nasal, buccal, rectal, vaginal, transdermal and the like can also be formulated from the particles made from the present invention.

[0046] Although a detailed description of the methods for making submicron-sized particles or other organic compound delivery vehicles (such as emulsions, microemulsions, liposomes, micelles etc.) is beyond the scope of the present invention, a brief description of a preferred method of making one preferred category of vehicle, namely, submicron-sized particles, such as nanoparticles, is provided below.

[0047] As shown in **FIG. 1**, (referred to Method A) the organic compound 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. One example of suitable solvent is N-methyl-2-pyrolidinone (NMP).

[0048] As set forth above, the organic compound delivery vehicles such as nanoparticles are typically combined with surfactants selected to prevent agglomeration, stabilize the compound and, preferably, act as an excipient for the compound. Thus, a second aqueous solvent is provided with one or more optional surface modifiers such as an anionic surfactant, a cationic surfactant, a nonionic surfactant or a biologically surface active molecule added thereto. U.S. patent application Ser. No. 10/703,395 (from which priority is claimed) describes suitable anionic, cationic and nonionic surfactants.

[0049] The first solution is then mixed or otherwise combined with the second aqueous solvent to define a pre-suspension of particles. Further steps include adding energy to the pre-suspension to form a suspension of submicron-sized particles. Additional details and alternative steps to this method of making submicron-sized particles are provided in U.S. Pat. No. 6,607,784, previously incorporated by reference, and will not be repeated here.

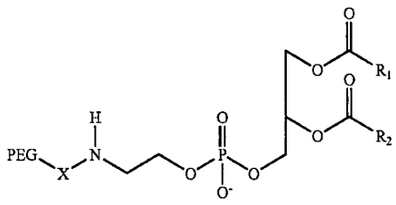
[0050] (In an alternative method of manufacturing submicron sized particles, the surfactant or combination of surfactants are initially combined with the first solution containing the organic compound. This method is shown in **FIG. 2** and referred to as Method B. In all other respects, the method is identical to Method A shown in **FIG. 1**).

[0051] Sub-micron sized particles with the surfactant system of the present invention may also be made by combining the organic compound, the block copolymer and the conjugated amphiphilic to form a mixture, adding a suitable grinding media to the mixture, and milling the mixture in the presence of the grinding media, as generally disclosed in U.S. Pat. No. 5,145,684, incorporated herein by reference.

[0052] Surfactant systems of the present invention include an amphiphilic surfactant such as a phospholipid or a ceramide. The phospholipids may be either synthetic or natural semisynthetic, salted or desalted, hydrogenated or partially hydrogenated. Phospholipids useful in the present invention may include fatty chains of at least 10 carbon atoms with varying degrees of saturation. The phospholipids may be diacyl phospholipids including dimyristoyl (14:0), dipalmitoyl (16:0), distearoyl (18:0), dioleolyl (18:1), or heterogeneous diacyl phospholipids, such as palmitoyl-stearoyl and the like. For example, the phospholipid may be a diacyl-glycero-phosphethanolamine such as: dimyristoyl-glycero-phosphoethanolamine (DMPE), dipalmitoyl-glycero-hosphoethanolamine (DPPE), distearoyl-glycero-phosphoethanolamine (DSPE), dioleolyl-glycero-phosphoethanolamine (DOPE) or the like. Other suitable phospholipids types include phosphatidylcholines, other phosphatidylethanolamines, phosphatidylserines, phosphatidylinositols, phosphatidylglycerols, phosphatidic acids, lysophospholipids, egg or soybean phospholipids or a combination thereof. As discussed below, the phospholipids or ceramides are preferably conjugated with a water-soluble or hydrophilic polymer.

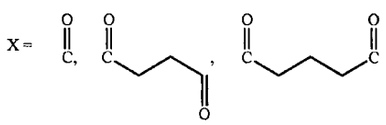
[0053] In one embodiment, the water-soluble or hydrophilic polymer conjugating to the phospholipids (or ceramide) may be polyethylene glycol (PEG). The molecular weight of the PEG may preferably be at least 200 to about 100,000 and more preferably, between about 200-50,000. Examples of suitable PEGs include, but are not limited to, PEG 350, PEG 550, PEG 750, PEG 1000, PEG 2000, PEG 3000, and PEG 5000. The PEGs may be simple PEGs, containing the repeating oxyethylene monomer, or further derivatized. A preferred PEG of the present invention is "mPEG," which is a methoxylated PEG. Conjugated lipids, of the type described above, are available from Avanti Polar Lipids, Inc. of Alabaster, Ala. Other hydrophilic polymer conjugates can also be used, e.g., dextran, hydroxypropyl methacrylate (HPMA), polyglutamate and the like, although PEG is preferred.

[0054] Thus, in a preferred embodiment, the surfactant combination includes a polyethylene glycol (PEG) i.e., "peglyated"-lipid conjugate. The conjugated PEG-lipids may be a PEG conjugated to phosphatidyl-ethanoalamine having the structure:

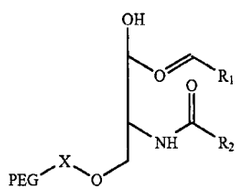


where: R_1=alkyl, alkenyl

[0055] R_2=alkyl, alkenyl

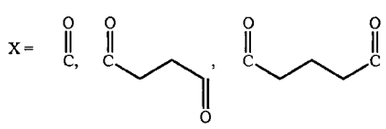


[0056] Alternatively, the PEG moiety may be linked to ceramide, and having the structure:



where: R_1=alkyl, alkenyl

[0057] R_2=alkyl, alkenyl



[0058] In accordance with the present invention, the conjugated amphiphile is combined with a second surface modifier to further stabilize the particles. The second surface modifier can be mixed into the water-miscible first solvent or the second solvent or both the water-miscible first solvent and the second solvent. The second surface modifier can be selected from anionic surfactants, cationic surfactants, nonionic surfactants and surface active biological modifiers as described in detail previously in this application.

[0059] A preferred second surface modifier is a nonionic surfactant that is a block copolymer of polyoxyethylene and polyoxypropylene or "poloxamer". Poloxamers come in a variety of types and are sometimes referred to and are sold under the trade name PLURONIC® from BASF Aktiengesellschaft. Examples of suitable poloxamers are Poloxamer 188 (Pluronic F-68), Poloxamer 124, Poloxamer 237, Poloxamer 338 and Poloxamer 407. Of these, Poloxamer 188 is most preferred.

[0060] A particularly preferred surfactant system for nanoparticles of different therapeutic, diagnostic agents or other organic compounds is one that combines the above-described Poloxamer 188 with the conjugated hydrophilic polymer/phospholipid mPEG-DSPE(2000). As previously described, Poloxamer 188 is a polyoxyethylene-polyoxypropylene copolymer that is a non-ionic surfactant with good physiological compatibility. Poloxamer 188 has the following chemical formula:

$$HO(CH_2CH_2O)_{80}(CH_3H_6O)_{27}(CH_2CH_2O)_{80}H$$

[0061] The phospholipid is a phosphatidyl ethanolamine of which a preferred example is DSPE such as 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine. The DSPE is preferably conjugated with the hydrophilic polymer polyethylene glycol moiety mPEG(2000)[methoxy(polyethyleneglycol)-2000]. The resulting polymer-phospholipid conjugate is 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine N-[methoxy(polyethyleneglycol)-2000], or mPEG-DSPE(2000).

[0062] The concentrations of surfactants employed in the composition will depend on the particular organic compound employed in the composition and its concentration, but will be in an amount sufficient to avoid substantial aggregation of the particles and effectively stabilize the system. In general, with higher concentrations of organic compound employed, typically higher concentrations of surfactants will also be employed. It is believed, however, that the surfactants will be employed in compositions that have organic compound concentrations of up to 50% (w/v), but more preferably about 0.05-20% (w/v)where the composition is provided as a "ready-to-use" liquid or as a frozen liquid. Thus, for the preferred organic compound concentration range (e.g., 0.05-20% w/v), the surfactants will be present in concentrations from about 0.01 to about 10% (w/v) of the composition and more preferably, from about 0.1-1.0% (w/v) of the composition. (The remainder of the composition will typically include water, salts and toxicity adjusters.)

[0063] The composition of the present invention may also be provided as a solid, including, but not limited to freeze-dried or lyophilized forms. When provided as a solid, the weight ratio of the organic compound to surfactant will typically be from about 1:1 to 20:1, with a weight ratio of 5:1 organic compound to the combined surfactant being preferred.

[0064] Regardless of the form in which the composition is provided (i.e., liquid "ready-to-use," frozen liquid, lyophilized or other solid form), relative to each other, the two surfactants will be present in an amount of about 10%-90% (w/w) of the conjugated conjugated amphiphile and 90-10% (w/w) of the block copolymer, relative to total weight of surfactant. More preferably, surfactant systems of the present invention include approximately 20%-40% (w/w) of the conjugated amphiphile and approximately 60%-80% (w/w) of the block copolymer, relative to total surfactant weight. In general, it is preferred that the ratio of block copolymer (e.g., Poloxamer) to conjugated lipid (e.g., MPEG-DSPE) be greater than 1:1, although an approxi-

mately 50/50% (w/w) mixture of the two surfactants, relative to total surfactant weight have also been shown to be effective.

[0065] The surfactant system described above is safe and biocompatible and provides good stability (and longer shelf life) for submicron sized particles. More particularly, in nanoparticles coated with this surfactant system, agglomeration of nanoparticles is substantially reduced. In addition, this system substantially prevents ripening of the nanoparticles (i.e., the dissolution and subsequent crystallization of smaller particles onto larger particles). Finally, nanoparticles coated with the surfactant system are less susceptible to scavenging by the reticuloendothelial system (RES) of the liver, a problem that is observed with other surfactant compounds. Avoiding scavenging by the RES allows the nanoparticle and, thus, the therapeutic or diagnostic agent to remain in circulation and offers better targeting of the agent in vivo.

[0066] The preferred surfactant system of mPEG-DSPE (2000) may be used with the organic compounds described in U.S. patent application Ser. No. 10/703,395 previously incorporated by reference and from which priority is claimed. The preferred surfactant system may also be used with diagnostic agents and other organic compounds previously described. In addition, the preferred surfactant system may be employed in other drug delivery vehicles such as micelles, liposomes, nanocapsules, emulsions, cubosomes, hexasomes, and microemulsions.

[0067] In a preferred method of preparing nanoparticles with the surfactant system of the present invention, the therapeutic agent is combined with a solvent of the type described above, such as, but not limited to NMP, to provide a first solvent solution. A second solvent solution including Poloxamer 188, the conjugated polymer/phospholipid mPEG-DSPE(2000) and, for example, a tonicity adjuster, such as sucrose, is separately prepared. The solutions are separately filtered. The first solvent solution is added to the second solvent solution, yielding a pre-suspension of microprecipitated agent coated with mPEG-DSPE(2000) and Poloxamer 188. The presuspension may then be subjected to an energy-addition step, as described above.

[0068] As demonstrated below, compositions of nanoparticles with the preferred surfactant system of a polyoxyethylene/polyoxypropylene block copolymer (e.g., Poloxamer) and conjugated phospholipid provide, in many respects, improved properties when compared to nanoparticles including only one of either the block copolymer or conjugated phospholipid. The improved properties typically include less aggregation and a smaller mean particle size, under normal as well as stressed conditions. Examples of stressed conditions include, but are not limited to, thermal cycling, repeated freeze-thaw cycling, agitation, and centrifugation. Stress testing methods for particles are well known in the art. Typical stress testing methods are described in detail in Novel Injectable Formulations of Insoluble Drugs, Pace et al., Pharm Tech, March 1999, pg 116-134. In addition, nanoparticles with the preferred surfactant system are less prone to scavenging by the RES of the liver, as compared to surfactant systems that do not include peglyated amphiphiles.

EXAMPLE 1A

Microprecipitation and Homogenization Processes for Making Paclitaxel Particles With mPEG-DSPE/Poloxamer Surfactant System

[0069] Example 1A:A solution of paclitaxel in NMP was precipitated in a surfactant solution containing 0.5% poloxamer 188 and 0.05% mPEG-DSPE (2000) (with 2% glycerin as a tonicity agent), at low temperature (<10° C.). The total suspension volume was 10 mL, with a drug concentration of 1% (w/v). High pressure homogenization was carried out immediately after precipitation, at a pressure of ~25,000 psi at a temperature of 40° C. After homogenization (20 minutes), particle size of the suspension was examined using light scattering. Mean particle size was 186 nm.

EXAMPLE 1B

[0070] A solution of paclitaxel in NMP was precipitated in a surfactant solution containing 0.5% w/v poloxamer 188 and 0.05% w/v mPEG-DSPE (2000) (with 2% w/v glycerin as a tonicity agent), at low temperature (<10° C.). The total suspension volume was 20 mL, with a drug concentration of 1% (w/v). High pressure homogenization was carried out immediately after precipitation, at a pressure of ~25,000 psi at a temperature of 40° C. After 30 minutes homogenization, particle size of the suspension was examined using light scattering. Mean particle size was 204 nm.

EXAMPLE 1C

[0071] A solution of paclitaxel in NMP was precipitated in a surfactant solution containing 0.5% poloxamer 188 and 0.05% mPEG-DSPE (2000) (with 2% glycerin as a tonicity agent), at low temperature (<10° C.) . The total suspension volume was 10 mL, with a drug concentration of 1% (w/v). High pressure homogenization was carried out immediately after precipitation, at a pressure of ~25,000 psi at a temperature of 70° C. After homogenization, particle size of the suspension was examined using light scattering. Mean particle size was 158 nm. About 45% of particles were under 150 nm.

EXAMPLE 1D

[0072] A solution of paclitaxel in NMP was precipitated in a surfactant solution containing 0.05% mPEG-DSPE (2000) (with 2% glycerin as a tonicity agent), at low temperature (<10° C.). The total suspension volume was 10 mL, with a drug concentration of 1% (w/v). High pressure homogenization was carried out immediately after precipitation, at a pressure of ~25,000 psi at a temperature of 40° C. After homogenization, particle size of the suspension was examined using light scattering. Mean particle size was 244 nm.

EXAMPLE 2

Dissolution Characteristics of Paclitaxel Submicron Particles with mPEG-DSPE/Poloxamer Surfactant System

[0073] Two formulations of paclitaxel particles prepared by the methods described in Example 1 were tested for their solubility by dissolution kinetics using % transmission at 400 nm as a measure for dissolution. The particles are not soluble if % transmission does not return to 100% after

addition of suspension. One formulation contains the surface modifiers poloxamer 188 (P188) and mPEG-DSPE (2000). The other formulation contains the surface modifier of mPEG-DSPE (2000) only. The results are shown in **FIG. 3**. In both cases, % transmission did not rise after the initial drop to about 60%, indicating that the particles do not dissolve.

EXAMPLE 3

Stability of Paclitaxel Submicron Particles With Mpeg-DSPE/Poloxamer Surfactant System Under Stressed Conditions and Upon Storage

[0074] Stability of the submicron paclitaxel particles prepared in Example 1A was tested using accelerated stress testing as well as storage at 5° C. for one month. As shown in **FIGS. 4 and 5**, the mean particle size and the 99th percentile both remained virtually unchanged. No aggregation was observed for the formulation either, even after all the stress tests. Aggregation was estimated by measuring particle size before and after 1 minute sonication and comparing the difference via the following equation:

$$\% \text{ Aggregation} = \frac{(P_{99} - P_{99S}) \times 100}{P_{99S}}$$

where P99 represents 99th percentile of the particle size distribution before sonication, and P99S represents 99th percentile of the particle size distribution after sonication.

[0075] The above results and those shown in **FIG. 4** were compared with those for nanoparticulate compositions including only DSPE-PEG 2000 (i.e., no Poloxamer), as reported in **FIG. 6**. As shown in **FIG. 6**, increased aggregation was observed when particles including only DSPE-PEG 2000 were subjected to thermal cycling, agitation or centrifugation.

EXAMPLE 4

Stability of Itraconazole Nanoparticles with Poloxamer 188/mPEG DSPE(2000) Surfactant System Under Stressed Conditions and Upon Storage

[0076] A surfactant solution containing 0.1% Poloxamer 188, 0.08% mPEG-DSPE(2000), and 9.25% sucrose was prepared. 0.505 g of itraconazole was added to the surfactant solution. The mixture was subjected to high shear mixing for 4 minutes using an Ultra-Turrax T8 mixer (setting 6) to form a presuspension. The presuspension was then homogenized in a C5 Avestin homogenizer for 8 minutes at 25,000 psi to form a nanosuspension. The mean particle size of the nanosuspension was 0.695 μm and 99% of the particles less than 1.651 μm.

[0077] This sample was subjected to various stress tests, including temperature cycling, centrifugation, agitation, and freezing. The results of the stress tests are shown in **FIG. 7**. Data is also included showing the effect of stress on nanoparticles treated with a surfactant solution including 0.1% Poloxamer 188 and 9.25% sucrose, but without mPEG-DSPE(2000) formulation. As seen in **FIG. 7**, no significant particle size increase or caking was found in the nanoparticles treated with the Poloxamer 188/mPEG-DSPE(2000) combination. In addition, this sample was placed on long-term stability at 5° C., 25° C., and 40° C. The results of the 3-month interval are shown in **FIG. 8**. As seen in **FIG. 7**, no significant increase in particle size was observed.

[0078] 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.

What is claimed:

1. A composition comprising an organic compound and at least two surfactants associated therewith, wherein relative to each other said surfactants comprise (a) approximately 90%-10% (w/w) of a block copolymer of polyoxyethylene and polyoxypropylene and (b) approximately 10%-90% (w/w) of an amphiphilic compound conjugated with a hydrophilic polymer.

2. The composition of claim 1 where said amphiphilic compound is selected from the group consisting of phospholipids and ceramides.

3. The composition of claim 1 wherein said block copolymer has the chemical formula: $HO(CH_2CH_2O)_{80}(CH_3H_6O)_{27}(CH_2CH_2O)_{80}H$.

4. The composition of claim 1 wherein said hydrophilic polymer comprises polyethylene glycol.

5. The composition of claim 3 wherein said polyethylene glycol is selected from the group consisting of PEG 2000, PEG 3000, and PEG 5000.

6. The composition of claim 4 wherein the molecular weight of said polyethylene glycol is between 200 and 50,000.

7. The composition of claim 1 wherein said phospholipid is 1,2-Distrearoyl-sn-glycero-3-phosphoethanolamine.

8. The composition of claim 1 wherein said phospholipid conjugated with said hydrophilic polymer comprises 1,2-Distrearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000].

9. The composition of claim 1 wherein said surfactant comprises the combination of Poloxamer 188 and mPEG-DSPE(2000).

10. The composition of claim 1 comprising a drug delivery vehicle selected from the group consisting of nanoparticles, emulsions, microemulsions, liposomes, cubosomes, hexasomes, and micelles.

11. The composition of claim 1 wherein said nanoparticle has a mean particle size of less than approximately 2 μm.

12. The composition of claim 1 comprising approximately 0.5%-20% (w/v) of said organic compound and approximately 0.1%-10% (w/v) of said at least two surfactants.

13. The composition of claim 12 comprising approximately 0.1%-1.0% (w/v) of said at least two surfactants.

14. The composition of claim 1 wherein said composition is provided in a form selected from the group consisting of liquid suspension, frozen liquid suspension and solid.

15. The composition of claim 1 wherein relative to each other, said surfactants comprise approximately 80%-60% (w/w) said block copolymer and approximately 20%-40% (w/w) of said amphiphilic compound conjugated with a hydrophilic polymer.

16. The composition of claim 1 wherein the ratio of block copolymer to said conjugated amphiphilic compound is greater than 1:1.

17. A composition comprising an organic compound and at least two surfactants associated therewith, wherein relative to each other said surfactants comprise (a) approximately 90%-10% (w/w) of the block copolymer $HO(CH_2CH_2O)_{80}(CH_3H_6O)_{27}(CH_2CH_2O)_{80}H$ and (b) approximately 10%-90% (w/w) of an amphiphilic compound conjugated with a hydrophilic polymer.

18. The composition of claim 17 wherein said amphiphilic compound is selected from the group consisting of phospholipids and ceramides.

19. The composition of claim 17 wherein said hydrophilic polymer comprises polyethylene glycol.

20. The composition of claim 17 wherein said polyethylene glycol is selected from the group consisting of PEG 2000, PEG 3000, and PEG 5000.

21. The composition of claim 17 wherein said phospholipid is 1,2-Distrearoyl-sn-glycero-3-phosphoethanolamine.

22. The composition of claim 17 comprising an organic compound delivery vehicle selected from the group consisting of nanoparticles, emulsions, microemulsions, liposomes, cubosomes, hexasomes, and micelles.

23. The composition of claim 17 comprising approximately 0.5%-20% (w/v) of said organic compound and approximately 0.%-10% (w/v) of said at least two surfactants.

24. The composition of claim 17 comprising approximately 01.1%-1.0% (w/v) of said at least two surfactants.

25. The composition of claim 17 wherein said composition is provided in a form selected from the group consisting of liquid suspension, frozen liquid suspension, and solid.

26. The composition of claim 17 wherein relative to each other, said surfactants comprise approximately 80%-60% (w/w) said block copolymer and approximately 20%-40% (w/w) of said amphiphilic compound conjugated with a hydrophilic polymer.

27. The composition of claim 17 wherein the ratio of block copolymer to said conjugated amphiphilic compound is greater than 1:1.

28. A composition comprising an organic compound and at least two surfactants associated therewith wherein relative to each other, said surfactants comprise (a) approximately 90%-10% (w/w) of a block copolymer of polyoxyethylene and polyoxypropylene and (b) between approximately 10%-90% (w/w) of a peglyated phosphatidyl ethanolamine.

29. The composition of claim 28 wherein said peglyated phosphatidyl ethanolamine comprises 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine conjugated with a polyethylene glycol.

30. The composition of claim 29 wherein said peglyated phosphatidyl ethanolamine comprises 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N[methoxy (polyethylene glycol)-2000].

31. The composition of claim 30 wherein said block copolymer has the chemical formula: $HO(CH_2CH_2O)_{80}(CH_3H_6O)_{27}(CH_2CH_2O)_{80}H$.

32. The composition of claim 28 comprising an organic compound delivery vehicle selected from the group consisting of nanoparticles, emulsions, microemulsions, liposomes, cubosomes, hexasomes, and micelles.

33. The composition of claim 28 wherein said nanoparticle has a mean particle size of less than approximately 2 μm.

34. The composition of claim 28 comprising approximately 0.5%-20% (w/v) of said organic compound and approximately 0.1%-10% (w/v) of said at least two surfactants.

35. The composition of claim 34 comprising approximately 0.1%-1.0% (w/v) of said at least two surfactants.

36. The composition of claim 28 wherein said composition is provided in a form selected from the group consisting of liquid suspension, frozen liquid suspension and solid.

37. The composition of claim 28 wherein relative to each other, said surfactants comprise approximately 80%-60% (w/w) said block copolymer and approximately 20%-40% (w/w) of said amphiphilic compound conjugated with a hydrophilic polymer.

38. The composition of claim 28 wherein the ratio of block copolymer to said conjugated amphiphilic compound is greater than 1:1.

39. A method for making particles of an organic compound, said particles having a mean particle size of about 10 nm to about 10 microns, comprising:

dissolving an organic compound in a water miscible solvent to form a first solution;

preparing an aqueous solution including at least two surfactants, wherein relative to each other said surfactants comprise approximately 10%-90% (w/w), of a block copolymer of polyoxyethylene and polyoxypropylene and approximately 90-10% (w/w), by weight of a hydrophilic polymer conjugated with phospholipids;

adding said first solution to said aqueous solution to form a presuspension; and

adding energy to said presuspension.

40. The method of claim 39 comprising preparing said aqueous solution wherein relative to each other, said surfactants comprise approximately 20%-40% (w/w) of said amphiphilic compound and between approximately 80%-60% (w/w) of said block copolymer.

41. The method of claim 39 comprising preparing an aqueous solution of the block copolymer: $HO(CH_2CH_2O)_{80}(CH_3H_6O)_{27}(CH_2CH_2O)_{80}H$ and a phospholipid conjugated with a hydrophilic polymer.

42. The method of claim 39 wherein said hydrophilic polymer comprises polyethylene glycol with a molecular weight of between 200 and 50,000.43. The method of claim 39 wherein said phospholipid comprises 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine.

44. The method of claim 39 wherein said phospholipid conjugated with said hydrophilic polymer comprises 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000].

45. The method of claim 40 comprising approximately 0.5%-20% (w/v) of said organic compound and approximately 0.1%-10% (w/v) of said at least two surfactants.

46. The method of claim 45 comprising approximately 0.1%-1.0% (w/v) of said at least two surfactants.

47. The method of claim 40 wherein said composition is provided in a form selected from the group consisting of liquid suspension, frozen liquid suspension and solid.

48. A method for making particles of an organic compound, the particles having mean particle size of about 10 nm to about 10 microns, comprising:

adding an organic compound and at least two surfactants, wherein relative to each other, said surfactants com-

prise approximately 90%-10% (w/w), of a block copolymer of polyoxyethylene and polyoxypropylene and approximately 10%-90% (w/w) of a hydrophilic polymer conjugated with a phospholipid to a milling apparatus and forming a mixture;

adding a suitable grinding media to the milling apparatus; and

milling the mixture in the presence of the grinding media.

49. The method of claim 48 comprising approximately 0.5%-20% (w/v) of said organic compound and approximately 0.%-10% (w/v) of said at least two surfactants.

50. The method of claim 49 comprising approximately 0.1%-1.0% (w/v) of said at least two surfactants.

51. The method of claim 48 wherein said composition is provided in a form selected from the group consisting of liquid suspension, frozen liquid suspension and solid.

52. A method of administering to a subject an effective amount of a composition comprising particles including an organic compound associated with at least two surfactants, the particles having a mean particle size of about 10 nm to about 10 microns, wherein relative to each other the surfactants comprise (a) approximately 90-10% (w/w), a block copolymer of polyoxyethylene and polyoxypropylene and (b) approximately 10%-90% (w/w) of an amphiphilic compound conjugated with a hydrophilic polymer.

53. The method of claim 52 wherein said composition comprises approximately 0.5%-20% (w/v) of said organic compound and approximately 0.1%-10% (w/v) of said at least two surfactants.

54. The method of claim 53 wherein said composition comprises approximately 0.1%-1.0% (w/v) of said at least two surfactants.

55. The method of claim 52 wherein said composition is provided in a form selected from the group consisting of liquid suspension, frozen liquid suspension, and solid.

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*** Conceptos Pagados ***

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				$300.000.00

SON: **TRESCIENTOS MIL PESOS MONEDA CORRIENTE***

Responsable: _____
-*****-
Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
No. 06-042082- -00008-0000
Fecha: 2012-03-15 16:43:54 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 523 RESPUESTA Folios: 35

Colo: 0873-841-020-6.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
- / -

Industria y Comercio
SUPERINTENDENCIA

RECIBO DE CAJA

No. 12 - 0024911

Bogotá D.C., Marzo 12 de 2012 - 07:29:37

RECIBIDO DE : CAVELIER ABOGADOS NI 860.041.367

*** Soporte del Pago ***

TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR.PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	157717630	09/03/2012	64.070.000.00

*** Conceptos Pagados ***

CANT. RENTISTICO	CONCEPTO	Vr.UNDITARIO	Vr.CONCEPTO
1 50005-01-01 SOLICITUDES	1641 S.DISTINTIVO INSCRIPCION AFECTACION (CB.NOMBRE, DOMICIL	300.000.00	300.000.00
			$300.000.00

SON: **TRESCIENTOS MIL PESOS MONEDA CORRIENTE***

Responsable: _____
-*******-

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Cod: 0587 3 - 841 - 020 - 6.