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5, Harish Mukherjee Road, Kolkata - 700 025, India
Tel: 91-33-2455 7484/85/86, Fax: 91-33-2455-7487/88, E-mail : cal@patentindia.com

The Controller of Patents
The Patent Office
New Delhi

7102
24/3/14

Via Hand
March 28, 2014

GOVT. OF INDIA PATENT OFFICE
D. No. 7102
28 MAR 2014
BAUDHIK SAMPADA BHAWAN
NEW DELHI

Kind Attention : **Dr. Nilanjana Mukherjee**
Asst. Controller of Patents & Designs

Re: Pre-grant Opposition u/s 25(1) against
Patent Application No. 2899/DELNP/2005
Abraxis BioSciences LLC. .....Applicant
Natco Pharma Ltd. .....Opponent
Our ref : PII 535

In connection with the captioned matter, we submit herewith the following documents:

i) Notice of Opposition on Form-7A (in duplicate)
ii) Representation (in duplicate) with Annexure A and Exhibits 1 to 3 (in duplicate)

We submit that as evident from the records of the captioned matter in the pre-grant opposition filed by the opponent in October 10, 2008, the amended claims were made available to the opponent on the eve of the opposition hearing scheduled on April 9, 2009. On the basis of such claims submissions on the ground of insufficiency were made during the hearing. The Ld. Controller was pleased to appreciate the applicability of such a ground on the basis of the amended claims and had returned a decision on such ground as well. The Hon'ble IPAB by an order dated January 20, 2014 has been pleased to set aside the order of the Ld. Controller directed the Ld. Controller to hear the matter afresh. The Hon'ble Board also held that as the the ground of opposition on "insufficiency" was not at all raised or pleaded by the opponent any finding on the said ground in the absence of pleading is unsustainable in law.

We respectfully submit that the insufficiency still stares in the complete specification and therefore the opponent has preferred to file the present representation under the grounds of Section 25(1)(g) and 25(1)(f) limited to Section 3(d).

We submit that the grounds taken in the present representation do not overlap and can therefore be adjudicated independently. We submit that we have no objection in the event the two representations are heard together.

The opponent as a matter of right under law files the opposition.

We request you to kindly take the opposition on record under intimation to us.

Yours faithfully,

Abhishek Sen

ORIGINAL

02899 DELNP 05

28 MAR 2014

FORM – 7A

THE PATENTS ACT, 1970

(39 OF 1970)

&

THE PATENTS RULES, 2003

REPRESENTATION FOR OPPOSITION TO GRANT OF PATENT

(See section 25 (1) and rule 55)

NATCO PHARMA LTD, is an Indian company having its registered office at H. No: 8-2-112/A/32, Road No. 2, Banjara Hills, Hyderabad – 500 033, Andhra Pradesh, hereby give representation by way of opposition to the grant of the patent in respect of Application No. **2899/DELNP/2005 Nationalised on June 29, 2005** made by Abraxis BioScience, LLC and published on 13/04/2007

The impugned patent application is opposed on the following grounds:

- that the subject matter of the claims is not an invention within the meaning of this Act or is not patentable under this Act **(Section 25(1)(f);**
- that the complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed **(Section 25 (1) (g))**

The address for service in India is S Majumdar & Co., 5 Harish Mukherjee Road, Kolkata-700 025, State of West Bengal. Phone 033-24557484, Fax: 033-24557487,

Dated this 27th day of March 2014

Abhishek Sen
(Opponent's Agent)

To
The Controller of Patents
The Patent Office,
At Delhi

DUPLICATE

FORM – 7A

02899 DELNP 05

28 MAR 2014

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The address for service in India is S Majumdar & Co., 5 Harish Mukherjee Road, Kolkata-700 025, State of West Bengal. Phone 033-24557484, Fax: 033-24557487,

Dated this 27th day of March 2014

Abhishek Sen
(Opponent's Agent)

To
The Controller of Patents
The Patent Office,
At Delhi

BEFORE THE CONTROLLER OF PATENTS

PATENT OFFICE

DELHI

02899 05

28 MAR 2014

ORIGINAL

PRE-GRANT OPPOSITION UNDER SECTION 25 (1) AGAINST PATENT

Application Number 2899/DELNP/2005 Nationalised On June 29, 2005

NATCO PHARMA LTD, is an Indian company having its registered office at H. No: 8-2-112/A/32, Road No. 2, Banjara Hills, Hyderabad – 500 033, Andhra Pradesh

---------- Opponent

Vs

ABRAXIS BIOSCIENCE, LLC ---------- Applicant

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Abhishek Sen

(Opponent's Agent)

The Controller of Patents
The Patent Office, Delhi

DUPLICATE

102899 05

BEFORE THE CONTROLLER OF PATENTS

PATENT OFFICE

DELHI

28 MAR 2014

PRE-GRANT OPPOSITION UNDER SECTION 25 (1) AGAINST PATENT

Application Number 2899/DELNP/2005 Nationalised On June 29, 2005

NATCO PHARMA LTD. is an Indian company having its registered office at H. No: 8-2-112/A/32, Road No. 2, Banjara Hills, Hyderabad – 500 033, Andhra Pradesh

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Abhishek Sen

(Opponent's Agent)

The Controller of Patents
The Patent Office, Delhi

ORIGINAL

BEFORE THE CONTROLLER OF PATENTS
DELHI

02899 DELNP 05

28 MAR 2014

In the matter of Section 25(1) of The Patents Act, 1970 as amended *by* The Patents (Amendment) Act 2005,

-And-

In the matter of The Patents Rules, 2003 as amended by The Patents Rules, 2006

-And-

IN THE MATTER of Patents Application No. 2899/DELNP/2005 by Abraxis BioScience, LLC, an American Company.....

.....Applicant

-And-

IN THE MATTER of opposition of the grant of a patent thereto by M/s. NATCO PHARMA LTD., an Indian Company at H. No: 8-2-112/A/32, Road No. 2, Banjara Hills, Hyderabad – 500 033, Andhra Pradesh

.....Opponent

REPRESENTATION OF OPPOSITION OF THE OPPONENT UNDER SECTION 25(1)

1. The Opponent, NATCO PHARMA LTD, is an Indian company having its registered office at H. No: 8-2-112/A/32, Road No. 2, Banjara Hills, Hyderabad – 500 033, Andhra Pradesh. The Opponent is engaged in the business of manufacturing and marketing of various pharmaceutical products.

2. An opposition was filed against the subject application under the grounds as stated under.

- Section 25(1)(b) - lack of novelty
- Section 25(1)(d) – prior use/prior public knowledge
- Section 25(1)(e) – lack of inventive step
- Section 25(1)(f) – not patentable under Section 3(e) and Section 3(i)
- Section 25(1)(i) – convention application not made within the 12 months from filing of first application

3. It is stated that amended claims were made available to the opponent on the eve of the hearing. On the basis of such claims submissions on the ground of insufficiency were made during the hearing. The Ld. Controller was pleased to appreciate the applicability of such a ground on the basis of the amended claims and had returned a decision on such ground as well.

4. An appeal was preferred by the applicant at the Hon'ble IPAB and the appeal has been disposed of by an order dated January 20, 2014 and the order of the Ld. Controller was set aside and the Ld. Controller was directed to hear the matter afresh.

5. With regard to the finding of the Ld. Controller on the ground of insufficiency the Hon'ble Board was pleased to hold as under -

13. The yet another contention of the learned counsel for the appellant is that the learned Assistant Controller has suo-moto taken an additional ground of opposition namely on

the ground of "insufficiency" and rendered finding in favour of the 4th respondent and such finding is erroneous in view of the absence of plea raised by the 4th respondent. It is pertinent to note that the learned Assistant Controller has formulated an additional ground of opposition on "insufficiency" under section 25(1)(g) of the Act and also rendered a finding in para 5.17 in the impugned order on the said ground. It is to be stated that the 4th respondent has not at all raised or pleaded the ground of opposition on "insufficiency" and as such any finding on the said ground in the absence of pleading is unsustainable in law."

6. The effect of such a finding by the Hon'ble IPAB is that the ground of insufficiency was not taken by the opponent herein in its representation and, therefore, the finding by the Ld. Controller is erroneous. The Hon'ble Board held that the 4th respondent has not at all raised or pleaded the ground of opposition on "insufficiency" and as such any finding on the said ground in the absence of pleading is unsustainable in law.

7. In view of the aforesaid findings the opponent is precluded from agitating the question of insufficiency in the hearing of the representation that was originally filed which did not contain the ground of insufficiency. The original representation was filed on the basis of the claims which were then on record and known to the opponent. Later, however, the claims were amended and the scope of the invention was altered by the applicant consequent on such amendments.

8. The claims as originally filed read as under.

1. A pharmaceutical composition comprising a pharmaceutical agent and a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier comprises albumin in an amount effective to reduce one or more side effects of administration of the pharmaceutical composition into a human, and wherein the pharmaceutically acceptable carrier comprises deferoxamine in an amount effective to inhibit microbial growth in the pharmaceutical composition.

2. The pharmaceutical composition of claim 1, wherein the pharmaceutical agent is selected from the group consisting of anticancer agents, anesthetics, antimicrotubule agents, agents to treat cardiovascular disorders, antihypertensives, anti- inflammatory agents, anti-arthritic agents, antiasthmatics, analgesics, vasoactive agents, immunosuppressive agents, antifungal agents, antiarrhythmic agents, antibiotics, and hormones.

3. The pharmaceutical composition of claim 2, wherein the pharmaceutical agent is selected from the group consisting of paclitaxel, docetaxel, taxanes, camptothecin, propofol, amiodarone, cyclosporine, rapamycin, amphotericin, liothyronine, epothilones, colchicines, thyroid hormones, vasoactive intestinal peptide, corticosteroids, melatonin, tacrolimus, mycophenolic acids, and derivatives thereof.

4. The pharmaceutical composition of claim 3, wherein the pharmaceutical agent is propofol.

5. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is a liquid and comprises from about 0. 1% to about 25% by weight of albumin.

6. The pharmaceutical composition of claim 5, wherein the pharmaceutical composition comprises about 0.5% to about 5% by weight of albumin.

7. The pharmaceutical composition of claim 5, wherein the pharmaceutical composition is dehydrated.

8. The pharmaceutical composition of claim 6, wherein the pharmaceutical composition is lyophilized.

9. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition comprises a mesylate salt of deferoxamine.

10. The pharmaceutical composition of claim 9, wherein the pharmaceutical composition is a liquid and comprises from about 0. 0001% to about 0.5% by weight of deferoxaminemesylate.

11. The pharmaceutical composition of claim 10, wherein the pharmaceutical composition comprises about 0. 1% by weight of deferoxaminemesylate

12. The pharmaceutical composition of claim 10, wherein the pharmaceutical composition is dehydrated.

13. The pharmaceutical composition of claim 12, wherein the pharmaceutical composition is lyophilized. l 14. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is an oil-in-water emulsion.

15. The pharmaceutical composition of claim 5, wherein the pharmaceutical agent is propofol.

16. The pharmaceutical composition of claim 10, wherein the pharmaceutical agent is propofol.

17. The pharmaceutical composition of claim 9, wherein the pharmaceutical agent is propofol, the propofol is present in an amount from about 0. 1% to about 5% by weight, the albumin is present in an amount from about 0. 1 % to about 25% by weight, and the deferoxaminemesylate is present in an amount from about 0.0001% to about 0.5% by weight.

18. A pharmaceutical composition comprising a pharmaceutical agent and a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier comprises albumin in an amount effective to reduce one or more side effects of administration of the pharmaceutical composition into a human, and wherein the pharmaceutically acceptable carrier comprises deferoxamine in an amount effective to inhibit oxidation in the pharmaceutical composition.

77. A pharmaceutical composition comprising a pharmaceutical agent and a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier comprises albumin in an amount effective to reduce one or more side effects of

administration of the pharmaceutical composition into a human, and wherein the ratio of albumin to pharmaceutical agent is about 18: 1 or less.

78. The pharmaceutical composition of claim 77, wherein the ratio of albumin to pharmaceutical agent in the pharmaceutical composition is about 12: 1 or less.

79. The pharmaceutical composition of claim 77, wherein the ratio of albumin to pharmaceutical agent in the pharmaceutical composition is about 9: 1 or less.

80. A pharmaceutical composition comprising a pharmaceutical agent and a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier comprises albumin in an amount effective to increase transport of the drug to the site of infirmity in a human, and wherein the ratio of albumin to pharmaceutical agent is about 18: 1 or less.

81. The pharmaceutical composition of claim 80, wherein the ratio of albumin to pharmaceutical agent in the pharmaceutical composition is about 12 : 1 or less.

82. The pharmaceutical composition of claim 80, wherein the ratio of albumin to pharmaceutical agent in the pharmaceutical composition is about 9: 1 or less.

83. The pharmaceutical composition of claim 80, wherein the infirmity is selected from the group consisting of cancer, arthritis, and cardiovascular disease.

84. The pharmaceutical composition of claim 1, wherein the ratio of albumin to pharmaceutical agent is about 18: 1 or less.

91. A pharmaceutical composition comprising a pharmaceutical agent and a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier comprises a protein in an amount effective to reduce one or more side effects of administration of the pharmaceutical composition into a human, and wherein the ratio of protein to pharmaceutical agent is about 18: 1 or less.

92. The pharmaceutical composition of claim 91, wherein the ratio of protein to pharmaceutical agent in the pharmaceutical composition is about 12: 1 or less.

93. The pharmaceutical composition of claim 91, wherein the ratio of protein to pharmaceutical agent in the pharmaceutical composition is about 9: 1 or less.

9. The claims as amended read as under (along with the reply statement)-

1.A sterile pharmaceutical composition comprising a water insoluble pharmaceutical agent of the kind such as herein described and a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier comprises albumin, wherein the ratio (w/w) of albumin to pharmaceutical agent in the pharmaceutical composition is 1:1 to 9:1, wherein the pharmaceutical composition comprises nanoparticles comprising the water insoluble pharmaceutical agent and albumin, and wherein the nanoparticles have a particle size of less than 200 nm.

2. The pharmaceutical composition as claimed in claim 1, wherein the albumin is human serum albumin.

3. The pharmaceutical composition as claimed in claims 1 or 2, wherein the pharmaceutical agent is selected from the group consisting of an anticancer agent, an anesthetic, an antimicrotubule agent, an agent to treat cardiovascular disorders, an antihypertensive, an anti-inflammatory agent, an anti-arthritic agent, an antiasthmatic, an analgesic, a vasoactive agent, an immunosuppressive agent, an antifungal agent, an antiarrhythmic agent, an antibiotic, and a hormone.

4. The pharmaceutical composition as claimed in claim 3, wherein the pharmaceutical agent is an anticancer agent.

5. The pharmaceutical composition as claimed in claim 3, wherein the pharmaceutical agent is an antibiotic.

6. The pharmaceutical composition as claimed in claim 3, wherein the pharmaceutical agent is an anti-inflammatory agent.

7. The pharmaceutical composition as claimed in claim 3, wherein the pharmaceutical agent is an immunosuppressive agent.

8. The pharmaceutical composition as claimed in claim 1, wherein the pharmaceutical agent is selected from the group consisting of taxane, camptothecin, propofol, amiodarone, cyclosporine, amphotericin, liothyronine, epothilone, colchicine, corticosteroid, melatonin, tacrolimus, mycophenolic acid and derivatives thereof.

9. The pharmaceutical composition as claimed in claim 7, wherein the pharmaceutical agent is a taxane.

10. The pharmaceutical composition as claimed in claim 9, wherein the taxane is paclitaxel.

11. The pharmaceutical composition as claimed in claim 9, wherein the pharmaceutical agent is docetaxel.

12. The pharmaceutical composition as claimed in claim 3, wherein the pharmaceutical agent is rapamycin.

13. The pharmaceutical composition as claimed in claim 3, wherein the pharmaceutical agent is IDN5390.

14. The pharmaceutical composition as claimed in any one of claims 1 to 13, wherein the ratio (w/w) of albumin to the water insoluble pharmaceutical agent in the pharmaceutical composition is less than 9:1.

15. The pharmaceutical composition as claimed in any one of claims 1 to 14, wherein the ratio (w/w) of albumin to the water insoluble pharmaceutical agent in the pharmaceutical composition is 5:1 or less.

16.The pharmaceutical composition as claimed in claim 15, wherein the ratio (w/w) of albumin to the water insoluble pharmaceutical agent in the pharmaceutical composition is 3:1 or less.

17. The pharmaceutical composition as claimed in any one of claims 1 to 16, wherein the composition comprises deferoxamine.

18. The pharmaceutical composition as claimed in any one of claims 1 to 17, wherein the composition comprises sterile saline."

10. Amended claims 1 to 12 (filed a day before the scheduled hearing under Section 25(1), i.e. April 8, 2009)

1. A sterile pharmaceutical composition comprising a water insoluble anticancer agent and a pharmaceutically acceptable carrier,wherein the pharmaceutically acceptable carrier comprises albumin wherein the ratio (w/w) of albumin to the anticancer agent in the pharmaceutical composition is 1:1 to 9:1 wherein the pharmaceutical composition comprises nanoparticles comprising the water insoluble anticancer agent and albumin, and wherein the nanoparticles have a particle size of less than 200nm.

2. The pharmaceutical compositions as claimed in claim 1, wherein the albumin is human serum albumin.

3. The pharmaceutical compositions as claimed in claim 1or 2, wherein the water insoluble anticancer agent is selected from the group consisting of taxane, camptothecin, liothyronine, epothilone, propofol, mycophenolic acid, colchicines corticosteroids, melatoninand derivatives thereof.

4 The pharmaceutical composition as claimed in claim 3, wherein the anti cancer agent is taxane.

5. The pharmaceutical composition as claimed in claim 4, wherein the taxane is paclitaxel.

6. The pharmaceutical composition as claimed in claim 4, wherein the taxane is docetaxel.

7. The pharmaceutical composition as claimed in claim 3, wherein the anticancer agent is IDN5390 or rapamycin.

8. The pharmaceutical composition as claimed in any one of claims 1 to 7, wherein the ratio (w/w) of albumin to the water insoluble anticancer agent in the pharmaceutical composition is less than 9:1.

9. The pharmaceutical composition as claimed in anvone of claims 1 to 8, wherein the ratio (w/w) of albumin to the water insoluble anticancer agent in the pharmaceutical composition is 5:1 or less.

10. The pharmaceutical composition as claimed in claim 9, wherein the ratio (w/w) of albumin to the water insoluble anticancer agent in the pharmaceutical composition is 3:1 or less.

11. The pharmaceutical composition as claimed in anyone of claims 1 to 10, wherein the composition comprises deferoxamine.

12. The pharmaceutical composition as claimed in any one of claims 1 to 11, wherein the composition comprises sterile saline.

11. Original claim 1 as filed and opposed

A pharmaceutical composition comprising a pharmaceutical agent and a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier comprises albumin in an amount effective to reduce one or more side effects of administration of the pharmaceutical composition into a human, and wherein the pharmaceutically acceptable carrier comprises deferoxamine in an amount effective to inhibit microbial growth in the pharmaceutical composition.

12. Amended claim 1 (circulated to the opponent alongwith the reply statement)

A sterile pharmaceutical composition comprising a water insoluble pharmaceutical agent of the kind such as herein described and a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier comprises albumin, wherein the ratio (w/w) of albumin to pharmaceutical agent in the pharmaceutical composition is 1:1 to 9:1, wherein the pharmaceutical composition comprises nanoparticles comprising the water insoluble pharmaceutical agent and albumin, and wherein the nanoparticles have a particle size of less than 200 nm.

13. Further amended claim 1 (filed a day before the scheduled hearing, i.e. on April 8, 2009)

A sterile pharmaceutical composition comprising a water insoluble anticancer agent and a pharmaceutically acceptable carrier,wherein the pharmaceutically acceptable carrier comprises albumin wherein the ratio (w/w) of albumin to the anticancer agent in the pharmaceutical composition is 1:1 to 9:1 wherein the pharmaceutical composition comprises nanoparticles comprising the water insoluble anticancer agent and albumin, and wherein the nanoparticles have a particle size of less than 200nm.

14. It is stated that upon comparing the original with the claim as amended, it is evident that amended claim 1 specifies ratio range of 1:1 to 9:1 as the ratio (w/w) of albumin to pharmaceutical agent, which is an anticancer agent, in the pharmaceutical composition while the original claim 1 did not indicate any ratio range. Also the amended claim 1 makes reference to nanoparticles comprising the water insoluble anticancer agent and albumin, having a particle size of less than 200 nm. This was not present in original claim 1. Significantly, the scope of the amended claim 1 has been altered by removing reference to an essential feature, namely deferoxamine, in an amount effective to inhibit microbial growth in the pharmaceutical composition. It is stated that, such removal of an essential feature from principal claim as amended is in total breach of the provisions of the Act.

15. It is stated that in the amended set of claims claim 1 is the only independent claim while all the subordinate claims are dependent from claim 1 and/or other claims. Therefore, the claim 1 is the broadest claim and all the other dependent claims are of narrower scope.

16. It is therefore stated that the amended set of claims are in total departure from the claims as originally filed and such amended claims do not find proper basis in the examples. It is stated that examples are essential for working an invention and enabling a person of ordinary skill to perform the invention. It is a prerequisite under the law to disclose enabling examples as part of the disclosure in a complete specification so as to enable public to carry out the invention after the expiry of the patent. Naturally such enabling examples are of particular relevance in a pharmaceutical invention.

17. It is stated that the amended claims as on record on the date of the hearing on April 9, 2009 before the Ld. Controller did not find support of the albumin to anticancer agent ratio of 9:1 or less which exhibits the alleged surprising properties of the claimed composition. It is also noteworthy that the Applicant's marketed product contains the albumin and paclitaxel in a 1:9 ratio. The marketed product is sold under the brand Abraxane which contains paclitaxel:albumin in the ratio of 1:9 and is free of solvent. The product is approved mainly for the treatment of metastatic breast cancer. Thus the specification fails to sufficiently describe the best embodiment of the claimed composition being the ratio of 9:1 (w/w) of albumin to Paclitaxel. It was shown during the hearing and established in a convincing manner that the Complete Specification does not sufficiently clearly describe the best embodiment namely, a composition having 9:1 (w/w) of albumin to Paclitaxel. A copy of the product label as available in public domain is annexed hereto and marked with the letter "A".

18. It was submitted that the claims as filed was the basis of the representation of the opponent which had the originally filed 93 claims and not the amended claims with a narrower ratio range and broadened scope vis-à-vis deletion of the essential antimicrobial, namely, deferoxamine.. Due to such flagrant breach of sufficiency of description the Ld. Controller had taken cognizance of the ground, the proceedings being pre-grant in nature. The Hon'ble Board having found that the consideration of the ground of insufficiency without support of pleadings was bad in law and puts the matter at rest.

19. It is stated and submitted that the insufficiencies still stare in the complete specification and the applicant in the present representation bases its case under the grounds of Section 25(1)(g) and 25(1)(f) limited to Section 3(d).

20. It is stated that the ground of 25(1)(g) was not taken at all in the first representation and that was one of the reasons why the Hon'ble Board considered it appropriate to set aside the first order of the Ld. Controller. Therefore, taking of the ground in the present representation does not overlap with the earlier opposition.

21. On the other hand, Section 25(1)(f) was taken in the earlier opposition but limited to the lack of patentability under Section 3(e) and 3(i). In the present representation Section 25(1)(f) is taken only limited to Section 3(d) which was not a part of the first opposition and there was no pleadings at all or even any mention of lack of patentability in view of Section 3(d).

22. It is stated that at the time of filing of the first representation in 2008 there was no judicial authority on the true scope of Section 3(d) and clarity is found in the Novartis decision which came into existence only in 2013. Also the affidavit of the applicant's expert, Dr. Anindya Sircar has been served on the opponent by the Ld. Controller most recently and such affidavit also prompts to raise the 3(d) ground.

23. It is stated that the grounds taken in the present representation do not overlap and can therefore be adjudicated independently. The opponent also has no objection in the event the two representations are heard together.

Section 25(1)(g) – INSUFFICIECNY OF DISCLOSURE

The opponent states that the complete specification contains 51 examples illustrating the preparation and demonstration of activity of several pharmaceutically active agents. In the amended claims the pharmaceutically active agents have been categorically limited to anticancer agents and therefore the opponent will dwell upon the examples which relate to anticancer agents, particularly Paclitaxel and the composition comprising paclitaxel and albumin in a ratio of 1:9 has been projected as the most significant embodiment of the invention.

24. It is stated that in Example 1 the proportion of albumin to Paclitaxel in the composition in w/w terms is not stated. If one tries to derive the proportion of albumin to Paclitaxel it comes to about 18:1. It is stated that the example provides the process for preparation in which the weight of Paclitaxel and volume of albumin is stated but the amounts in weight terms of

Paclitaxel and albumin are not stated. A person of average skill in the art who will prepare the composition will not know what amount of Paclitaxel and albumin should be taken to obtain 9:1 (w/w) of albumin to Paclitaxel so as to arrive at the product claimed. Such ratio is also the best embodiment as the applicant markets a composition comprising said ratio as apparent from the annexed label. Therefore, example 1 does not sufficiently and clearly describe the invention of the method by which it is to be performed.

The other examples pertaining to compositions of paclitaxel have been listed below.

Example 15 – Demonstration of preclinical pharmacokinetics and pharmacodynamics of a pharmaceutical composition comprising albumin and paclitaxel

Example 16 – Demonstration of reduced side effects and reduced toxicity associated with pharmaceutical compositions comprising paclitaxel and albumin.

Example 17 – Demonstration of the clinical effects of a pharmaceutical composition comprising paclitaxel and albumin in humans.

Example 18 – Demonstration of enhanced preclinical efficacy using a pharmaceutical composition comprising albumin and paclitaxel.

Example 19 – Demonstration of enhanced clinical efficacy using a pharmaceutical composition comprising albumin and paclitaxel administered intra-arterially.

Example 32 – Demonstration of intrapulmonary delivery of a pharmaceutical composition comprising paclitaxel and albumin (ABI-007).

Example 33 - Demonstration of an investigation of Aerotech II and Pari nebulizers for pulmonary delivery of pharmaceutical compositions comprising paclitaxel and albumin.

Example 36 – Demonstration of oral delivery of a pharmaceutical composition comprising paclitaxel and albumin (ABI-007).

Example 37 – Demonstration of improved penetration of paclitaxel into red blood cells and tumor cells upon administration of a pharmaceutical composition comprising paclitaxel and albumin.

Example 38 – Demonstration of the safety of a pharmaceutical composition comprising paclitaxel and albumin administered to mice.

Example 39 – Demonstration of a novel paclitaxel transport mechanism across microvessel endothelial cells (EC) for paclitaxel-albumin compositions.

Example 40 – Demonstration of an increase in endothelial transcytosis of pharmaceutical compositions comprising paclitaxel and albumin as compared to Taxol.

Example 41 – Demonstration of improved endothelial cell (EC) binding by pharmaceutical compositions comprising paclitaxel and albumin as compared to Taxol.

Example 42 – Demonstration of improved albumin binding by pharmaceutical compositions comprising paclitaxel and albumin as compared to Taxol.

Example 43 – Demonstration of increased transfer of paclitaxel to albumin for pharmaceutical compositions comprising paclitaxel and albumin as compared to Taxol.

Example 44 – Demonstration of that the glycoprotein receptor gp60 is responsible for binding and transcytosis of albumin-paclitaxel.

Example 45 – Demonstration of that increasing amounts of albumin can compete with binding of paclitaxel.

Example 46 – Demonstration of that lower amounts of albumin in the inventive pharmaceutical composition results in stable compositions.

Example 47 – Demonstration of a pharmaceutical composition comprising albumin and paclitaxel having a high albumin to paclitaxel ratio.

Example 48 – Demonstration of a pharmaceutical composition comprising albumin and paclitaxel having a low albumin to paclitaxel ratio.

Example 49 – Demonstration of a pharmaceutical composition comprising albumin and paclitaxel having an intermediate albumin to paclitaxel ratio.

Example 50 – Demonstration of the treatment of rheumatoid arthritis in an animal model with an albumin-paclitaxel composition.

Example 52 – Demonstration of the use of albumin-paclitaxel compositions to treat cardiovascular restenosis.

32. It is stated that although examples 15-19, 32-33, 36-46 exemplify compositions comprising a paclitaxel and albumin; they fail to disclose the ratio of albumin and paclitaxel. Accordingly it can be presumed that such ratio is 1:18 as the initial preparation of the composition as put forth in example 1 contained a ratio of 1:18 (paclitaxel:albumin).

33. It is stated that examples 45 and 46 specifically disclose that increasing amount of albumin can compete with binding of paclitaxel and low amounts of albumin in the composition results in stable compositions respectively. The ratio of albumin to paclitaxel in these two examples can be presumed to be 18:1 since it is the only ratio which has been enabled by way of example 1 and no other ratio has been enabled in examples preceding example 45. Therefore, lesser toxicity as well as interference with binding of active can be solved by a paclitaxel to albumin ratio of 18:1 as seen in said examples.

34. The opponent states that examples 47 and 48 illustrate compositions having a high albumin to paclitaxel ratio, i.e., 27:1 and a low ratio of 4.5:1 respectively. It is also stated that the results observed with regard to activity in these examples are same, thus failing to establish the superiority of the ratio range claimed in amended claim 1.

It is stated that the applicant's marketed product contains the paclitaxel and albumin in a 1:9 ratio and sold under the brand name of Abraxane. The marketing approval of such product was obtained in 2005 and such product has been in circulation for the past about 9 years. This goes to prove beyond doubt that the most effective product out of a large range of products claimed in the patent and in diverse ratios the product Abraxane is the best and therefore used by the applicant.

It is stated that examples 47 and 48, where a high ratio of albumin to Paclitaxel 27:1 and a low ratio of 4.5:1 are said to be used, make same observations pertaining to reduced toxicity. Therefore, even these examples fail to support the surprising effects of the so-called inventive ratio range of albumin to Paclitaxel of 1:1 to 1:9.

35. Example 48 is the sole example demonstrating a ratio of paclitaxel to albumin within the range claimed in the amended claims.It is reiterated that none of the examples enable the ratio as asserted and claimed to be associated with the surprising effects of the composition. Therefore, none of the examples forming part of the specification on the date of filing the application sufficiently and clearly describe the invention, particularly the amounts of paclitaxel and albumin to be taken to yield a product having a ratio range claimed in amended claim 1. The complete specification thus fails to disclose sufficiently and clearly the best method or mode of performing the invention as stated in the preceding paragraphs.

36. It is stated that the complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed thus rendering it insufficient and warranting rejection.

Section 25(1)(f) : NOT PATENTABLE UNDER SECTION 3(d)

37. According to the disclosure of the invention in the impugned application the preamble paragraph categorically states that the inventions pertains to pharmaceutical composition comprising pharmaceutically active agents, which composition have the effect of reducing certain undesirable side effects compared with available formulation of similar drugs. Therefore, it is clear that the invention aims at providing a composition with reduced side effect using the same pharmaceutically active agents and not a composition which has significantly enhanced therapeutic efficacy.

38. It is stated that the invention as disclosed covers a large number of actives, however, the patentee after various studies have received approval of a pharmaceutical substance being a combination of albumin and Paclitaxel in the specific ratio of 9:1. It is stated that there is no example in the patent specification covering the best embodiment of the invention some of the studies involving albumin and Paclitaxel examples are found in examples 15 to 19 and 32 to 51.

While there are about 26 examples covering the combination of albumin and Paclitaxel the best combination which has received regulatory approval is not enabled. Example 15 although makes reference to albumin Paclitaxel pharmaceutical combination but does not make any indication about the ratio of the carrier and the active which, according to the specification, may be in the range of 0.01 : 1 to about 100 : 1. Therefore, example 15 is silent about albumin and Paclitaxel ratio and therefore cannot be related to the data provided in the affidavit of Dr. Anindya Sircar dated April 9, 2009. From example 15 it is apparent that the sole object of the study embodied in this example is to show the advantage of albumin Paclitaxel combination over the paclitaxel compositions containing cremophor (commercially available as Taxol), although the fact remains that the acknowledged prior art namely, US Patent No.5439686 discloses the combination of albumin and Paclitaxel. The disclosure in the impugned application seeks to cover a very wide range of actives for treating indications of very wide nature as apparent from paragraph 16 of the specification. The range from anti-cancer agents to aneasthetic to antibiotic to analgesic to vasoactive agents the same paragraph towards the end makes reference to Paclitaxel based compositions namely Taxol®. Eventually the marketing approval is in respect of a composition comprising Paclitaxel as the active and the combination is characterized by the ratio of albumin: Paclitaxel at 9: 1. The opponent states that the present invention is a mere discovery of a new form of a known substance without resulting in significantly different properties with regard to efficacy.

38. It is stated that the applicant's claim a particular ratio range of the anticancer agent to albumin, being 1:1 to 1:9, which is associated with alleged surprising effects. It is stated US 5439686, US 6096331, and WO 00/71079 teach compositions comprising drug and albumin in a ratio ranging from 1:9 to 1:13.3. In particular example 4, 2 and example 1 of the referred patents respectively. Thus, all the applicant has done is to also cover a reduced band of range from 1:1 to 1:9. Admittedly, Paclitaxel as an active is known to be efficacious for the treatment of breast cancer. Also the approved drug under the impugned application is also efficacious for the treatment of breast cancer. It is stated that the activity of the drug flows from the active substance namely Paclitaxel. The applicant has not shown that the composition of the approved drug or any composition claimed in the impugned patent differ significantly in properties with regard to efficacy from the known efficacy. Therefore, for a different dosage form of the combination of

the drug and albumin no patent can be granted as no enhanced efficacy is shown to have been achieved. US 5439686, US 6096331, and WO 00/71079 are annexed hereto as Exhibit 1, 2 and 3 respectively.

41. Further, it is clear beyond doubt that the claimed composition is essentially a protein-pharmaceutical agent combination which goes and binds to the receptor causing the transport of the pharmaceutical agent to a cell. Therefore, the protein acts as a transport agent or vehicle to make the pharmaceutical agent available to the same in a manner which is better than using the pharmaceutical agent alone and without the protein.

42. The alleged new properties include of the claimed composition are as under.

1. It is sterile.
2. Reduces or eliminates oxidation of pharmaceutical formulations to prevent drug destabiliation.
3. Reduces one or more side effects of administration of the pharmaceutical composition into a human.
4. Increases the transport of a pharmaceutical agent to a cell in vitro or in vivo by combining said agent with a protein, where the binding of the protein-pharmaceutical agent combination with the said receptor causes the transport to occur.

43. However the applicant has failed to demonstrate the significance of such properties with regard to efficacy in view of the known substance, as disclosed in the afore mentioned prior patents, namely, US5439686, US 6096331, and WO 00/71079.

44. It is stated that the claimed composition is a mere discovery of a new form of a known substance without any significant difference in properties with regard to efficacy, therefore clearly violative the provision of Section 3(d) from one of its aspects.

45. It is therefore stated that the claims of the impugned application warrant rejection solely under this ground.

ORIGINAL 102899 05

28 MAR 2014

RELIEF SOUGHT

The opponent states that it has established and made out a case on each of the aforesaid grounds of opposition and pray to the Ld. Controller for the following relief(s):

1) Take on record the present representation;

2) Leave to file evidence;

3) Forward copy of reply of applicant and evidence if any and any amendments filed;

4) Leave to file a replication to the reply of the applicant and evidence;

5) Leave to make further submissions in case the applicant makes any amendments in the claims;

6) Grant of hearing to the opponent;

7) Rejection of the patent application in toto;

8) Such other relief or reliefs as the Controller may deem appropriate.

Dated this 27th day of March 2014

Abhishek Sen

Opponent's Agent
S. Majumdar & Co.

To,
The Controller of Patents,
Patent Office,
Delhi

Abraxane®

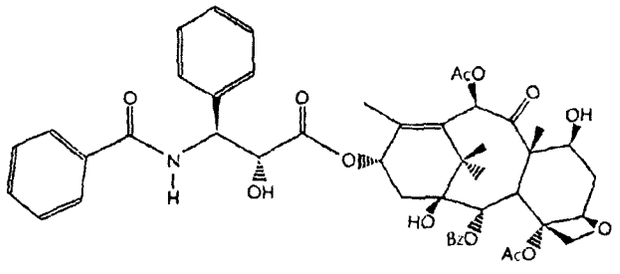
(nanoparticle albumin-bound paclitaxel)

PRODUCT INFORMATION

NAME OF THE MEDICINE

ABRAXANE (nanoparticle albumin-bound paclitaxel) 100 mg powder for injection (suspension).

The empirical formula for Paclitaxel is $C_{47}H_{51}NO_{14}$. The CAS Number for paclitaxel is 33069-62-4. The chemical name for paclitaxel is 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2*R*,3*S*)-N-benzoyl-3-phenylisoserine. Paclitaxel has the following chemical structure:



DESCRIPTION

ABRAXANE (nanoparticle albumin-bound paclitaxel) 100 mg powder for injection (suspension) is an albumin nanoparticle form of paclitaxel with a mean particle size of approximately 130 nanometres. Paclitaxel exists in the nanoparticles in a non-crystalline, amorphous state. Each vial of ABRAXANE contains paclitaxel and human albumin in the ratio of 1:9. The paclitaxel is contained within nanoparticles that consist of an average of 76% paclitaxel bound to 24% human albumin. Following administration, the nanoparticle rapidly dissociates to form albumin-bound paclitaxel and free paclitaxel with a ratio of 94:6.

ABRAXANE is supplied as a white to yellow, sterile, lyophilised powder in a 50 mL glass vial.

Each single-use vial contains the following:

Paclitaxel 100 mg

Excipients:
Human albumin solution (containing sodium, sodium octanoate and N-acetyl tryptophan).

The reconstituted medicinal product contains approximately 85 mg sodium per vial. ABRAXANE is free of solvents.

The active agent in ABRAXANE is paclitaxel, a natural product with antitumour activity. Paclitaxel is obtained from Taxus media.

Paclitaxel is a white to off-white crystalline powder with a molecular weight of 853.91. It is highly lipophilic, insoluble in water.

PHARMACOLOGY

Paclitaxel, the active pharmaceutical ingredient in ABRAXANE, is an antimicrotubule agent that promotes the assembly of microtubules from tubulin dimers and stabilises microtubules by preventing depolymerisation.

This stability results in the inhibition of the normal dynamic reorganisation of the microtubule network that is essential for vital interphase and mitotic cellular functions. Paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

Pharmacokinetics

Absorption and Distribution: The pharmacokinetics of total paclitaxel following 30- and 180-minute infusions of ABRAXANE at dose levels of 80 to 375 mg/m² were determined in clinical studies.

Following intravenous administration of ABRAXANE, paclitaxel plasma concentrations declined in a biphasic manner, the initial rapid decline representing distribution to the peripheral compartment and the slower second phase representing drug elimination. The terminal half-life was about 27 hours.

AUCs were dose proportional in the range 80 to 300 mg/m² and the pharmacokinetics of paclitaxel for ABRAXANE were independent of the duration of administration.

The pharmacokinetic parameters in patients with metastatic breast cancer are summarised in Table 1. The large volume of distribution indicates extensive extravascular distribution and/or tissue binding of paclitaxel.

Table 1: Summary of Pharmacokinetic Parameters in Patients with Metastatic Breast Cancer

(n = 12; ABRAXANE Dose and Regimen: 260 mg/m² q3w* over 30 min)					
	Cmax (ng/mL)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (h*ng/mL)	Clearance (L/h/m²)	Volume of Distribution (L/m²)
Single dose mean	18,700	27.4	17,900	15.2	632

* Once every 3 weeks

In vitro studies of binding to human serum proteins, using paclitaxel concentrations ranging from 0.1 to 50 µg/mL, indicate that between 89% to 98% of drug is bound, although studies specifically investigating protein binding with this formulation of paclitaxel were not conducted. The presence of cimetidine, ranitidine, dexamethasone, or diphenhydramine did not affect protein binding of paclitaxel.

Metabolism and Excretion: After a 30-minute infusion of 260 mg/m² doses of ABRAXANE, the mean values for cumulative urinary recovery of unchanged drug (4%) indicated extensive non-renal clearance. Less than 1% of the total administered dose was excreted in urine as the metabolites 6α-hydroxypaclitaxel and 3'-p-hydroxypaclitaxel. Faecal excretion was approximately 20% of the total dose administered. Hepatic metabolism has been demonstrated in animals.

The pharmacokinetics of paclitaxel may also be altered *in vivo* as a result of interactions with compounds that are substrates, inducers, or inhibitors of CYP2C8 and/or CYP3A4 (see **PRECAUTIONS: Interactions with other medicines**). The effect of renal or hepatic dysfunction on the disposition of ABRAXANE has not been investigated.

Possible interactions of paclitaxel with concomitantly administered medications have not been formally investigated.

CLINICAL TRIALS

Metastatic Breast Carcinoma

In a multi-centre trial, patients with metastatic breast cancer were randomised to receive paclitaxel every 3 weeks, either in a solvent-based form at 175 mg/m^2 in a 3-hour intravenous infusion (n=227) or as ABRAXANE 260 mg/m^2 in a 30-minute intravenous infusion (n=233). Premedication was given with solvent-based paclitaxel to prevent hypersensitivity. The treatments were not blinded. Two patients randomised to solvent-based paclitaxel and four to ABRAXANE did not receive any treatment.

Sixty-four percent of patients had impaired performance status (ECOG 1 or 2) at study entry; 79% had visceral metastases; and 76% had > 3 sites of metastases.

Fourteen percent of the patients had not received prior chemotherapy; 27% had received chemotherapy in the adjuvant setting only, 40% in the metastatic setting only, and 19% in both metastatic and adjuvant settings.

Fifty-nine percent received study drug as second or greater than second-line therapy. Seventy-seven percent of the patients had been previously exposed to anthracyclines.

Table 2 shows the results of the intent-to-treat analysis.

Table 2: Results for overall response rate, median time to disease progression, and progression-free survival as assessed by the investigator

Efficacy variable	ABRAXANE (260 mg/m^2) (n=233)	Solvent-based paclitaxel (175 mg/m^2) (n=227)	p-value Ratio [95% CI]
Response rate[a] (%)	32.6	18.5	≤0.001[b] 1.76 [1.27, 2.45]
Time to disease progression (months)*	Median 5.3	Median 3.8	0.003[c] 0.73 [0.59, 0.90]
Progression Free Survival (months)*	Median 5.2	Median 3.8	0.003[c] 0.73 [0.60, 0.90]
Survival (months)*	Median 15.0	Median 12.7	0.35[c] 0.90 [0.73, 1.12]

* This data is based on Clinical Study Report: CA012-0 Addendum dated Final (23 March 2005)

[a] Response rate is the sum of the complete and partial response rates assessed according to RECIST criteria

[b] Cochran-Mantel-Haenszel test

[c] Log-rank test

INDICATIONS

ABRAXANE is indicated for the treatment of metastatic carcinoma of the breast after failure of anthracycline therapy.

CONTRAINDICATIONS

ABRAXANE should not be used in patients who have baseline neutrophil counts of < 1.5 x 10^9/L.

In patients who have exhibited hypersensitivity reactions to paclitaxel or albumin, patients should not be treated with ABRAXANE.

ABRAXANE is contraindicated during pregnancy and lactation.

PRECAUTIONS

ABRAXANE should be administered under the supervision of a physician experienced in the use of cancer chemotherapeutic agents. Appropriate management of complications is possible only when adequate diagnostic and treatment facilities are readily available.

Note: An albumin form of paclitaxel may substantially affect a drug's functional properties relative to those of drug in solution. DO NOT SUBSTITUTE ABRAXANE WITH OTHER PACLITAXEL FORMULATIONS.

Haematology

Bone marrow suppression (primarily neutropenia) is dose dependent and a dose limiting toxicity. ABRAXANE therapy should not be administered to patients with baseline neutrophil counts of less than 1.5 x 10^9/L. In order to monitor the occurrence of myelotoxicity, it is recommended that frequent peripheral blood cell counts be performed on all patients receiving ABRAXANE. Patients should not be retreated with subsequent cycles of ABRAXANE until neutrophils recover to a level >1.5 x 10^9/L and platelets recover to a level >100 x 10^9/L. In the case of severe neutropenia (<0.5 x 10^9/L for seven days or more) during a course of ABRAXANE therapy, a dose reduction for subsequent courses of therapy is recommended (see **DOSAGE and ADMINISTRATION**).

Neuropathy

Sensory neuropathy occurs frequently with ABRAXANE. The occurrence of grade 1 or 2 sensory neuropathy does not generally require dose modification. If grade 3 sensory neuropathy develops, treatment should be withheld until resolution to grade 1 or 2 followed by a dose reduction for all subsequent courses of ABRAXANE.

Hepatic Impairment

Patients with hepatic impairment may be at increased risk of toxicity, particularly from myelosuppression, and such patients should be closely monitored for development of profound myelosuppression. The use of ABRAXANE has not been formally studied in patients specifically with hepatic impairment. Patients with severe hepatic impairment should not be treated with ABRAXANE. The appropriate dose regime in patients with mild to moderate hepatic impairment is unknown.

Effects on Fertility

Administration of albumin-bound paclitaxel to male rats on a weekly basis for 11 weeks prior to mating with untreated female rats was associated with testicular atrophy / degeneration and reduced fertility accompanied by decreased pregnancy rates and increased loss of embryos in mated females. Testicular atrophy / degeneration has also been observed in single dose toxicology studies in rodents administered albumin-bound paclitaxel particles at 6 mg/kg (54 mg/m^2) and dogs administered 8.75 mg/kg (175 mg/m^2).

Use in Pregnancy

Category D

ABRAXANE may cause foetal harm when administered to a pregnant woman. Administration of albumin-bound paclitaxel to female rats on gestation days 7 to 17 daily at doses of 1 mg/kg (6 mg/m^2: less than the daily maximum recommended human dose on an AUC basis) caused embryo- and foetotoxicity, as indicated by intrauterine mortality, increased resorptions, reduced numbers of live foetuses, reduction in foetal body weight and increase in foetal abnormalities. Foetal abnormalities included skeletal and soft tissue malformations, such as eye bulge, folded retina, and dilation of brain ventricles.

There are no adequate and well-controlled studies in pregnant women using ABRAXANE. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the foetus. Women of childbearing potential should be advised to avoid becoming pregnant while receiving treatment with ABRAXANE.

Like other genotoxic cytostatics, ABRAXANE can have genotoxic effects. Male patients treated with ABRAXANE are advised not to father a child during and up to six months after treatment.

Use in lactation

It is not known whether paclitaxel is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in breastfeeding infants, it is recommended that breastfeeding be discontinued when receiving ABRAXANE therapy.

Paediatric Use
The safety and effectiveness of ABRAXANE in paediatric patients have not been evaluated.

Use in Elderly
Of the 229 patients in the randomised study who received ABRAXANE, 13% were at least 65 years of age and < 2% were 75 years or older. No toxicities occurred notably more frequently among elderly patients at least 65 years of age who received ABRAXANE.

Carcinogenicity
The carcinogenic potential of ABRAXANE has not been studied.

Genotoxicity
Paclitaxel has been shown to be clastogenic *in vitro* (chromosome aberrations in human lymphocytes) and *in vivo* (micronucleus test in mice). Paclitaxel was not mutagenic in the Ames test or the CHO/HGPRT gene mutation assay.

Interactions with Other Medicines
No drug interaction studies have been conducted with ABRAXANE.

Drugs Metabolised in the Liver
The metabolism of paclitaxel is catalysed, in part, by cytochrome P450 isoenzymes CYP2C8 and CYP3A4. Therefore, caution should be exercised when administering ABRAXANE concomitantly with medicines known to inhibit (e.g. erythromycin, ketoconazole, fluoxetine, imidazole antifungals, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir) or induce (e.g. rifampicin, carbamazepine, phenytoin, efavirenz, nevirapine) either CYP2C8 or CYP3A4.

Possible interactions of albumin-bound paclitaxel with concomitantly administered medications have not been formally investigated in patients. *In vitro* studies using rat and human liver slices and liver microsomes have shown that the metabolism of paclitaxel is inhibited by a large number of drugs, including CYP2C8 and CYP3A4 substrates, and quinidine, PEG-35 castor oil, quercetin, clozapine, morin, and resveratrol.

Effects on Laboratory Tests
Interactions with laboratory tests have not been established.

ADVERSE EFFECTS

Table 3: Frequency[a] of Important Treatment Emergent Adverse Effects in the Randomised Study on an Every-3-Weeks Schedule

	Percent of Patients	
	ABRAXANE 260/30min[b] (n=229)	Paclitaxel Injection 175/3h[c,d] (n=225)
Bone Marrow		
Neutropenia		
< 2.0 x 10^9/L	80	82
< 0.5 x 10^9/L	9	22
Thrombocytopenia		
< 100 x 10^9/L	2	3
< 50 x 10^9/L	<1	<1
Anemia		
< 110 g/L	33	25
< 80 g/L	1	<1
Infections	24	20
Febrile Neutropenia	2	1
Bleeding	2	2
Hypersensitivity Reaction[e]		
All	4	12
Severe[f]	0	2

Table 3: Frequency[a] of Important Treatment Emergent Adverse Effects in the Randomised Study on an Every-3-Weeks Schedule, Continued

	Percent of Patients	
	ABRAXANE 260/30min[b] (n=229)	Paclitaxel Injection 175/3h[c,d] (n=225)
Cardiovascular		
Vital Sign Changes[g]		
Bradycardia	<1	<1
Hypotension	5	5
Severe Cardiovascular Events[f]	3	4
Abnormal ECG		
All patients	60	52
Patients with Normal Baseline	35	30
Respiratory		
Cough	7	6
Dyspnea	12	9
Sensory Neuropathy		
Any Symptoms	71	56
Severe Symptoms[f]	10	2
Myalgia / Arthralgia		
Any Symptoms	44	49
Severe Symptoms[f]	8	4
Asthenia		
Any Symptoms	47	39
Severe Symptoms[f]	8	3
Fluid Retention / Edema		
Any Symptoms	10	8
Severe Symptoms[f]	0	<1
Gastrointestinal		
Nausea		
Any Symptoms	30	22
Severe Symptoms[f]	3	<1
Vomiting		
Any Symptoms	18	10
Severe Symptoms[f]	4	1
Diarrhoea		
Any Symptoms	27	15
Severe Symptoms[f]	<1	1
Mucositis		
Any Symptoms	7	6
Severe Symptoms[f]	<1	0

Table 3: Frequency[a] of Important Treatment Emergent Adverse Effects in the Randomised Study on an Every-3-Weeks Schedule, Continued

	Percent of Patients	
	ABRAXANE 260/30min[b] (n=229)	Paclitaxel Injection 175/3h[c,d] (n=225)
Alopecia	90	94
Hepatic (Patients with Normal Baseline)		
Bilirubin Elevations	7	7
Alkaline Phosphatase Elevations	36	31
AST (SGOT) Elevations	39	32
Injection Site Reaction	<1	1

[a] Based on worst grade.

[b] ABRAXANE dose in mg/m²/duration in minutes.

[c] paclitaxel injection dose in mg/m²/duration in hours.

[d] paclitaxel injection pts received premedication.

[e] Includes treatment-related events related to hypersensitivity (e.g., flushing, dyspnea, chest pain, hypotension) that began on a day of dosing.

[f] Severe events are defined as at least grade 3 toxicity.

[g] During study drug dosing.

Table 4 lists adverse effects associated with the administration of ABRAXANE to patients from studies in which ABRAXANE has been administered as a single agent at any dose in any indication (N = 789).

The frequency of undesirable effects listed in Table 4 is defined using the following convention:

Very common (≥1/10); common (≥ 1/100, <1/10); uncommon (≥1/1,000, <1/100); rare (≥1/10,000, <1/1,000); very rare (< 1/10,000).

Table 4: Adverse Effects Reported with ABRAXANE at Any Dose in Clinical Trials

Infections and infestations	Common: Infection, urinary tract infection, folliculitis, upper respiratory tract infection, candidiasis, sinusitis Uncommon: Oral candidiasis, nasopharyngitis, cellulitis, herpes simplex, viral infection, pneumonia, catheter-related infection, fungal infection, herpes zoster, injection site infection, respiratory tract infections
Neoplasms benign, malignant and unspecified	Uncommon: Metastatic pain, tumour necrosis
Blood and lymphatic system disorders	Very Common: Neutropenia, anaemia, leukopenia, thrombocytopenia, lymphopenia, bone marrow suppression Common: Febrile neutropenia
Immune system disorders	Uncommon: Hypersensitivity Rare: Severe hypersensitivity
Metabolism and nutrition disorders	Very common: Anorexia Common: Dehydration, decreased appetite, hypokalaemia Uncommon: Hypophosphataemia, fluid retention, hypoalbuminaemia, polydipsia, hyperglycaemia, hypocalcaemia, hypoglycaemia, hyponatraemia
Psychiatric disorders	Common: Insomnia, depression, anxiety Uncommon: Restlessness
Nervous system disorders	Very Common: Peripheral neuropathy, neuropathy, hypoaesthesia, paraesthesia. Common: Sensory neuropathy, peripheral sensory neuropathy, headache, dysgeusia, dizziness, peripheral motor neuropathy, ataxia, sensory disturbance, somnolence. Uncommon: Polyneuropathy, areflexia, dyskinesia, hyporeflexia, neuralgia, sensory loss, syncope, postural dizziness , neuropathic pain, tremor
Eye disorders	Common: Increased lacrimation , blurred vision, dry eye, keratoconjunctivitis sicca, madarosis Uncommon: Eye irritation, eye pain, abnormal vision, reduced visual acuity, conjunctivitis, visual disturbance, eye pruritus, keratitis
Ear and labyrinth disorders	Common: Vertigo Uncommon: Ear pain, tinnitus
Cardiac disorders	Common: Arrhythmia, chest pain, dyspnea, edema, flushing, hypotension, hypertension, pulmonary emboli, pulmonary thromboembolism, supraventricular tachycardia, Tachycardia Rare: bradycardia, cardiac arrest, congestive heart failure, left ventricular dysfunction
Vascular disorders	Common: Flushing, hot flushes, hypertension, lymphoedema Uncommon: Hypotension, peripheral coldness, orthostatic hypotension Rare: Thrombosis
Respiratory, thoracic and mediastinal disorder	Common: Dyspnoea, epistaxis, pharyngolaryngeal pain, cough, rhinitis, rhinorrhoea Uncommon: Productive cough, exertional dyspnoea, sinus congestion, decreased breath sounds, pleural effusion, allergic rhinitis, hoarseness, nasal congestion, nasal dryness, wheezing, pulmonary emboli, pulmonary thromboembolism, radiation pneumonitis Rare: Interstitial pneumonitis

Gastrointestinal disorders	Very Common: Nausea, diarrhoea, vomiting, constipation, stomatitis, mucositis Common: Abdominal pain, abdominal distension, upper abdominal pain, dyspepsia, gastrooesophageal reflux disease, oral hypoaesthesia Uncommon: Dysphagia, flatulence, glossodynia, dry mouth, gingival pain, loose stools, oesophagitis, lower abdominal pain, mouth ulceration, oral pain, rectal haemorrhage
Hepatobiliary disorders	Uncommon: Hyperbilirubinaemia, hepatomegaly
Skin and subcutaneous tissue disorders	Very Common: Alopecia, rash Common: Nail disorder, pruritus, dry skin, erythema, nail pigmentation/discolouration, skin hyperpigmentation, onycholysis, nail changes Uncommon: Nail bed tenderness, urticaria, skin pain, photosensitivity reaction, pigmentation disorder, pruritic rash, skin disorder, hyperhidrosis, onychomadesis, erythematous rash, generalised rash, dermatitis, night sweats, maculo-papular rash, vitiligo, hypotrichosis, nail discomfort, generalised pruritus, macular rash, papular rash, skin lesion, swollen face
Musculoskeletal and connective tissue disorders	Very Common: Arthralgia, myalgia Common: Pain in extremity, bone pain, back pain, muscle cramps, limb pain Uncommon: Chest wall pain, muscular weakness, neck pain, groin pain, muscle spasms, musculoskeletal pain, flank pain, limb discomfort, muscle weakness
Renal and urinary disorders	Uncommon: Dysuria, pollakiuria, haematuria, nocturia, polyuria, urinary incontinence
Reproductive system and breast disorders	Uncommon: Breast pain
General disorders and administration site conditions	Very Common: Fatigue, asthenia, pyrexia Common: Peripheral oedema, mucosal inflammation, pain, rigors, oedema, weakness, decreased performance status, chest pain, influenza-like illness, malaise, lethargy, hyperpyrexia Uncommon: Chest discomfort, abnormal gait, swelling, injection site reaction
Investigations	Common: Decreased weight, increased alanine aminotransferase, increased aspartate aminotransferase, decreased haematocrit, decreased red blood cell count, increased body temperature, increased gamma-glutamyltransferase, increased blood alkaline phosphatase Uncommon: Increased blood pressure, increased weight, increased blood lactate dehydrogenase, increased blood creatinine, increased blood glucose, increased blood phosphorus, decreased blood potassium, increased bilirubin
Injury, poisoning and procedural complications	Uncommon: Contusion Rare: radiation recall phenomenon, radiation pneumonitis

POST-MARKETING EXPERIENCE

Table 5: Adverse Reactions Reported during Post-Marketing (by MedDRA System Organ Class and Preferred Term in Alphabetical Order)

System Organ Class	Preferred Term
Blood and Lymphatic System Disorders	Pancytopenia
Nervous System Disorders	Cranial nerve palsies, vocal cord paresis
Skin/Subcutaneous Disorders	Erythema, maculo-papular rash, palmar-plantar erythrodysaesthesiae in patients previously exposed to capcitabine, photosensitivity reaction, Stevens-Johnson syndrome, toxic epidermal necrolysis
Injury, Poisoning and Procedural Complications	Radiation recall phenomenon
General Disorders and Administration Site Conditions	Extravasation
Immune System Disorders	Severe hypersensitivity

DOSAGE AND ADMINISTRATION

The reconstituted suspension is milky and homogenous without visible particles.

ABRAXANE should be administered under the supervision of a physician experienced in the use of chemotherapeutic agents.

ABRAXANE is for single use in one patient only. Discard any residue.

No premedication to prevent hypersensitivity reactions is required prior to administration of ABRAXANE.

The recommended regimen for ABRAXANE is 260 mg/m^2 administered intravenously over 30 minutes every 3 weeks.

Dose Adjustment

Patients who experience severe neutropenia (neutrophil <500 cells/mm^3 for a week or longer) or severe sensory neuropathy during ABRAXANE therapy should have dosage reduced to 220 mg/m^2 for subsequent courses of ABRAXANE. For recurrence of severe neutropenia or severe sensory neuropathy, additional dose reduction should be made to 180 mg/m^2. ABRAXANE should not be administered until neutrophil counts recover to >1.5 x 10^9/L. For grade 3 sensory neuropathy hold treatment until resolution to grade 1 or 2, followed by a dose reduction for all subsequent courses of ABRAXANE.

Missed Dose

ABRAXANE is administered every three weeks. In the event that the next scheduled dose is missed, dosing should occur as soon as possible, consistent with good medical practice, after the missed dose.

Hepatic Insufficiency

No data are currently available to recommend dosage alterations in patients with mild to moderate hepatic impairment. Patients with severe hepatic impairment should not be treated with albumin-bound paclitaxel.

Patients with impaired renal function

Studies in patients with impaired renal function have not been performed and there is insufficient data to permit dosage recommendations in this patient population.

Preparation and Administration Precautions

ABRAXANE is a cytotoxic anticancer drug and, as with other potentially toxic paclitaxel compounds, caution should be exercised in handling ABRAXANE. The use of gloves is recommended. If ABRAXANE (lyophilised cake or reconstituted suspension) contacts the skin, wash the skin immediately and thoroughly with soap and water. Following topical exposure to paclitaxel, events may include tingling, burning and redness. If ABRAXANE contacts mucous membranes, the membranes should be flushed thoroughly with water.

Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration. Limiting the infusion of ABRAXANE to 30 minutes, as directed, reduces the likelihood of infusion-related reactions.

Each mL of the reconstituted formulation will contain 5 mg/mL paclitaxel.

Calculate the exact total dosing volume of 5 mg/mL suspension required for the patient:
Dosing volume (mL) = Total dose (mg)/5 (mg/mL)

Do not mix any other drugs with the ABRAXANE infusion.

Preparation for Intravenous Administration

ABRAXANE is supplied as a sterile lyophilised powder for reconstitution before use. **AVOID ERRORS, READ ENTIRE PREPARATION INSTRUCTIONS PRIOR TO RECONSTITUTION.**

Vial Size	Volume of Diluent to be Added to Vial	Approximate Available Volume	Nominal Concentration per mL
50 mL	20 mL	20 mL	5 mg/mL

1. Aseptically, reconstitute each vial by injecting 20 mL of 0.9% Sodium Chloride Injection.
2. Slowly inject the 20 mL of 0.9% Sodium Chloride Injection *over a minimum of 1 minute, using the sterile syringe to direct* the solution flow onto the INSIDE WALL OF THE VIAL.
3. DO NOT INJECT the 0.9% Sodium Chloride Injection directly *onto the lyophilised cake as this will result in foaming.*
4. Once the injection is complete, allow the vial to sit for a minimum of 5 minutes to ensure proper wetting of the lyophilised cake/powder.
5. Gently swirl and/or invert the vial slowly for at least 2 minutes until complete dissolution of any cake/powder occurs. Avoid generation of foam.
6. If foaming or clumping occurs, stand solution for at least 15 minutes until *foam subsides.*

The reconstituted sample should be milky and homogenous without visible particulates. If particulates or settling are visible, the vial should be **gently** inverted again to ensure complete resuspension prior to use. Discard the reconstituted suspension if precipitates are observed. Discard any unused portion.

Inject the appropriate amount of reconstituted ABRAXANE into an empty, sterile, polyvinyl chloride (PVC) or non-PVC type IV bag. The use of specialised DEHP-free solution containers or administration sets is not necessary, but may be used if desired to prepare or administer ABRAXANE infusions. The use of an in line filter is not recommended.

Parenteral drug products should be inspected visually for particulate matter and discolouration prior to administration whenever solution and container permit.

Retain in the original package to protect from bright light.

Unopened vials of ABRAXANE are stable until the date indicated on the package when stored between 20°C to 25°C, in the original package. Neither freezing nor refrigeration adversely affects the stability of the product.

Stability of reconstituted suspension in the vial

Reconstituted ABRAXANE should be used immediately, but may be refrigerated at 2°C to 8°C (36°F to 46°F) for a maximum of 8 hours if necessary. If not used immediately, each vial of reconstituted suspension should be replaced in the original carton to protect it from bright light. Discard any unused portion.

Stability of the reconstituted suspension in the infusion bag

The suspension for infusion prepared as recommended in an infusion bag should be used immediately. To reduce microbiological hazard, use as soon as practicable after dilution. If storage is necessary, hold at 2 - 8°C for not more than 8 hours

Handling and Disposal

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published. There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

OVERDOSAGE

There is no known antidote for ABRAXANE overdosage. The primary anticipated *complications of overdosage would consist of bone marrow suppression, sensory* neurotoxicity, and mucositis.

PRESENTATION AND STORAGE CONDITIONS

Pack

ABRAXANE is supplied as a white to yellow, sterile, lyophilised cake for reconstitution in a *50 mL clear glass vial with a latex free stopper, individually packaged in a carton.* Each single use vial contains 100 mg of paclitaxel and 900 mg of human albumin. ABRAXANE is free of solvents.

After reconstitution with 20 mL of 0.9% Sodium Chloride Injection each millilitre (mL) of reconstituted suspension contains 5 mg of paclitaxel.

Pack Size

1 single vial in a carton.

Storage

Store the vials in original cartons below 25°C. Protect from light.

NAME AND ADDRESS OF SPONSOR

In Australia:

Abraxis BioScience Australia Pty Ltd
Level 1, 711 High Street
Kew East, Victoria 3102
Australia

Distributed by Specialised Therapeutics Australia Pty Ltd
Ph: 1300 798 820
Fax: 1800 798 829
www.specialisedtherapeutics.com.au

In New Zealand:

Pharmacy Retailing (NZ) Limited
trading as Healthcare Logistics
PO Box 62027
Mt Wellington AUCKLAND
New Zealand
Ph: (09) 918 5100
Fax: (09) 918 5101

POISON SCHEDULE

S4

DATE OF APPROVAL

Approved by the Therapeutic Goods Administration on 29 September 2008
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Safety Related Notification made: 09 September 2011

Approved by Medsafe on 15 July 2010

Abraxis BioScience Australia Pty Ltd
is an indirect subsidiary of
Celgene Corporation.

STA1450511

Specialised Therapeutics
AUSTRALIA

PO BOX 250 East Kew
Victoria 3102 Australia
Telephone 1300 798 820
Facsimile 1800 798 829
ABN 73 124 031 241

Abraxane®
(nanoparticle albumin-bound paclitaxel)

EXHIBIT-1

US005439686A

United States Patent [19]

Desai et al.

[11] Patent Number: 5,439,686

[45] Date of Patent: Aug. 8, 1995

[54] METHODS FOR IN VIVO DELIVERY OF SUBSTANTIALLY WATER INSOLUBLE PHARMACOLOGICALLY ACTIVE AGENTS AND COMPOSITIONS USEFUL THEREFOR

[75] Inventors: Neil P. Desai; Patrick Soon-Shiong; Paul A. Sandford, all of Los Angeles; Mark W. Grinstaff, Pasadena, all of Calif.; Kenneth S. Suslick, Champaign, Ill.

[73] Assignee: VivoRx Pharmaceuticals, Inc., Santa Monica, Calif.

[21] Appl. No.: 23,698

[22] Filed: Feb. 22, 1993

[51] Int. Cl.[6] A61K 9/48

[52] U.S. Cl. 424/451; 424/465; 424/489

[58] Field of Search 424/451, 465, 450, 439; 260/403

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Primary Examiner—Thurman K. Page
Assistant Examiner—Benston, Jr. William E.
Attorney, Agent, or Firm—Stephen E. Reiter; Pretty, Schroeder, Brueggemann & Clark

[57] ABSTRACT

In accordance with the present invention, there are provided compositions for the in vivo delivery of substantially water insoluble pharmacologically active agents (such as the anticancer drug taxol) in which the pharmacologically active agent is delivered in a soluble form or in the form of suspended particles. In particular, the soluble form may comprise a solution of pharmacologically active agent in a biocompatible dispersing agent contained within a protein walled shell. Alternatively, the protein walled shell may contain particles of taxol. In another aspect, the suspended form comprises particles of pharmacologically active agent in a biocompatible aqueous liquid.

17 Claims, No Drawings

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METHODS FOR IN VIVO DELIVERY OF SUBSTANTIALLY WATER INSOLUBLE PHARMACOLOGICALLY ACTIVE AGENTS AND COMPOSITIONS USEFUL THEREFOR

The present invention relates to in vivo delivery of substantially water insoluble pharmacologically active agents (e.g., the anticancer drug taxol). In one aspect, the agent is dispersed as a suspension suitable for administration to a subject, or is dissolved in a suitable biocompatible liquid. In another aspect, water insoluble pharmacologically active agents (e.g., taxol) are encased in a polymeric shell formulated from a biocompatible polymer. The polymeric shell contains particles of pharmacologically active agent, and optionally a biocompatible dispersing agent in which pharmacologically active agent can be either dissolved or suspended.

BACKGROUND OF THE INVENTION

Taxol is a natural product first isolated from the Pacific Yew tree, *Taxus brevifolia*, by Wani et al. [J. Am. Chem. Soc. Vol. 93:2325 (1971)]. Among the antimitotic agents, taxol, which contains a diterpene carbon skeleton, exhibits a unique mode of action on microtubule proteins responsible for the formation of the mitotic spindle. In contrast with other antimitotic agents such as vinblastine or colchicine, which prevent the assembly of tubulin, taxol is the only plant product known to inhibit the depolymerization process of tubulin, thus preventing the cell replication process.

Taxol, a naturally occurring diterpenoid, has been shown to have significant antineoplastic and anticancer effects in drug-refractory ovarian cancer. Taxol has shown excellent antitumor activity in a wide variety of tumor models such as the B16 melanoma, L1210 leukemias, MX-1 mammary tumors, and CS-1 colon tumor xenografts. Several recent press releases have termed taxol as the new anticancer wonder-drug. Indeed, taxol has recently been approved by the Federal Drug Administration for treatment of ovarian cancer. The poor aqueous solubility of taxol, however, presents a problem for human administration. Indeed, the delivery of drugs that are inherently insoluble or poorly soluble in an aqueous medium can be seriously impaired if oral delivery is not effective. Accordingly, currently used taxol formulations require a cremaphore to solubilize the drug. The human clinical dose range is 200–500 mg. This dose is dissolved in a 1:1 solution of ethanol:cremaphore and diluted to one liter of fluid given intravenously. The cremaphore currently used is polyethoxylated castor oil.

In phase I clinical trials, taxol itself did not show excessive toxic effects, but severe allergic reactions were caused by the emulsifiers employed to solubilize the drug. The current regimen of administration involves treatment of the patient with antihistamines and steroids prior to injection of the drug to reduce the allergic side effects of the cremaphore.

In an effort to improve the water solubility of taxol, several investigators have modified its chemical structure with functional groups that impart enhanced water-solubility. Among them are the sulfonated derivatives [Kingston et al., U.S. Pat. No. 5,059,699 (1991)], and amino acid esters [Mathew et al., J. Med. Chem. Vol. 35:145–151 (1992)] which show significant biological activity. Modifications to produce a water-soluble derivative facilitate the intravenous delivery of taxol dissolved in an innocuous carrier such as normal saline. Such modifications, however, add to the cost of drug preparation, may induce undesired side-reactions and/or allergic reactions, and/or may decrease the efficiency of the drug.

Microparticles and foreign bodies present in the blood are generally cleared from the circulation by the 'blood filtering organs', namely the spleen, lungs and liver. The particulate matter contained in normal whole blood comprises red blood cells (typically 8 microns in diameter), white blood cells (typically 6–8 microns in diameter), and platelets (typically 1–3 microns in diameter). The microcirculation in most organs and tissues allows the free passage of these blood cells. When microthrombii (blood clots) of size greater than 10–15 microns are present in circulation, a risk of infarction or blockage of the capillaries results, leading to ischemia or oxygen deprivation and possible tissue death. Injection into the circulation of particles greater than 10–15 microns in diameter, therefore, must be avoided. A suspension of particles less than 7–8 microns, is however, relatively safe and has been used for the delivery of pharmacologically active agents in the form of liposomes and emulsions, nutritional agents, and contrast media for imaging applications.

The size of particles and their mode of delivery determines their biological behavior. Strand et al. [in *Microspheres-Biomedical Applications*, ed. A. Rembaum, pp 193–227, CRC Press (1988)] have described the fate of particles to be dependent on their size. Particles in the size range of a few nanometers (nm) to 100 nm enter the lymphatic capillaries following interstitial injection, and phagocytosis may occur within the lymph nodes. After intravenous/intraarterial injection, particles less than about 2 microns will be rapidly cleared from the blood stream by the reticuloendothelial system (RES), also known as the mononuclear phagocyte system (MPS). Particles larger than about 7 microns will, after intravenous injection, be trapped in the lung capillaries. After intraarterial injection, particles are trapped in the first capillary bed reached. Inhaled particles are trapped by the alveolar macrophages.

Pharmaceuticals that are water-insoluble or poorly water-soluble and sensitive to acid environments in the stomach cannot be conventionally administered (e.g., by intravenous injection or oral administration). The parenteral administration of such pharmaceuticals has been achieved by emulsification of the oil solubilized drug with an aqueous liquid (such as normal saline) in the presence of surfactants or emulsion stabilizers to produce stable microemulsions. These emulsions may be injected intravenously, provided the components of the emulsion are pharmacologically inert. U.S. Pat. No. 4,073,943 describes the administration of water-insoluble pharmacologically active agents dissolved in oils and emulsified with water in the presence of surfactants such as egg phosphatides, pluronics (copolymers of polypropylene glycol and polyethylene glycol), polyglycerol oleate, etc. PCT International Publication No. WO85/00011 describes pharmaceutical microdroplets of an anaesthetic coated with a phospholipid such as dimyristoyl phosphatidylcholine having suitable dimensions for intradermal or intravenous injection.

Protein microspheres have been reported in the literature as carriers of pharmacological or diagnostic agents. Microspheres of albumin have been prepared by either heat denaturation or chemical crosslinking. Heat denatured microspheres are produced from an emulsi-

fied mixture (e.g., albumin, the agent to be incorporated, and a suitable oil) at temperatures between 100° C. and 150° C. The microspheres are then washed with a suitable solvent and stored. Leucuta et al. [International Journal of Pharmaceutics Vol. 41:213-217 (1988)] describe the method of preparation of heat denatured microspheres.

The procedure for preparing chemically crosslinked microspheres involves treating the emulsion with glutaraldehyde to crosslink the protein, followed by washing and storage. Lee et al. [Science Vol. 213:233-235 (1981)] and U.S. Pat. No. 4,671,954 teach this method of preparation.

The above techniques for the preparation of protein microspheres as carriers of pharmacologically active agents, although suitable for the delivery of water-soluble agents, are incapable of entrapping water-insoluble ones. This limitation is inherent in the technique of preparation which relies on crosslinking or heat denaturation of the protein component in the aqueous phase of a water-in-oil emulsion. Any aqueous-soluble agent dissolved in the protein-containing aqueous phase may be entrapped within the resultant crosslinked or heat-denatured protein matrix, but a poorly aqueous-soluble or oil-soluble agent cannot be incorporated into a protein matrix formed by these techniques.

BRIEF DESCRIPTION OF THE INVENTION

Thus it is an object of this invention to deliver pharmacologically 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 emulsifiers 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 microparticles suspended in a suitable biocompatible liquid.

It is yet another object of the invention to deliver pharmacologically active agents enclosed within a polymer shell which is further suspended in a biocompatible liquid.

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 discovered that substantially water insoluble pharmacologically active agents can be delivered in the form of microparticles 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 polyethoxylated 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 compositions is their propensity to produce allergic side effects.

The delivery of substantially water insoluble pharmacologically active agents in the form of a microparticulate suspension allows some degree of targeting to organs such as the liver, lungs, spleen, lymphatic circulation, and the like, through the use of particles of varying size, and through administration by different routes. The invention method of delivery further allows the administration of substantially water insoluble pharmacologically active agents employing a much smaller volume of liquid and requiring greatly reduced administration time relative to administration volumes and times required by prior art delivery systems (e.g., intravenous infusion of approximately one to two liters of fluid over a 24 hour period are required to deliver a typical human dose of 200-400 mg of taxol).

In accordance with another embodiment of the present invention, we have developed compositions useful for in vivo delivery of substantially water insoluble pharmacologically active agents. Invention compositions comprise substantially water insoluble pharmacologically active agents (as a solid or liquid) contained within a polymeric shell. The polymeric shell is a biocompatible polymer, crosslinked by the presence of disulfide bonds. The polymeric shell, containing substantially water insoluble pharmacologically active agents therein, is then suspended in a biocompatible aqueous liquid for administration.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided compositions for in vivo delivery of a substantially water insoluble pharmacologically active agent,

wherein said agent is a solid or liquid substantially completely contained within a polymeric shell,

wherein the largest cross-sectional dimension of said shell is no greater than about 10 microns,

wherein said polymeric shell comprises a biocompatible polymer which is substantially crosslinked by way of disulfide bonds, and

wherein said polymeric shell containing pharmacologically active agent therein is suspended in a biocompatible aqueous liquid.

As used herein, the term "in vivo delivery" refers to delivery of a pharmacologically active agent by such routes of administration as oral, intravenous, subcutaneous, intraperitoneal, intrathecal, intramuscular, inhalational, topical, transdermal, suppository (rectal), pessary (vaginal), and the like.

As used herein, the term "micron" refers to a unit of measure of one one-thousandth of a millimeter.

As used herein, the term "biocompatible" describes a substance that does not appreciably alter or affect in any adverse way, the biological system into which it is introduced.

Key differences between the pharmacologically active agents contained in a polymeric shell according to the invention and protein microspheres of the prior art are in the nature of formation and the final state of the protein after formation of the particle, and its ability to carry poorly aqueous-soluble or substantially aqueous-insoluble agents. In accordance with the present invention, the polymer (e.g., a protein) is selectively chemically crosslinked through the formation of disulfide bonds through, for example, the amino acid cysteine that occurs in the natural structure of a number of proteins. A sonication process is used to disperse a dispersing agent containing dissolved or suspended pharmacologically active agent into an aqueous solution of a biocompatible polymer bearing sulfhydryl or disulfide groups (e.g., albumin) whereby a shell of crosslinked polymer is formed around fine droplets of non-aqueous medium. The sonication process produces cavitation in the liquid that causes tremendous local heating and results in the formation of superoxide ions that crosslink the polymer by oxidizing the sulfhydryl residues (and/or disrupting existing disulfide bonds) to form new, crosslinking disulfide bonds.

In contrast to the invention process, the prior art method of glutaraldehyde crosslinking is nonspecific and essentially reactive with any nucleophilic group present in the protein structure (e.g., amines and hydroxyls). Heat denaturation as taught by the prior art significantly and irreversibly alters protein structure. In contrast, disulfide formation contemplated by the present invention does not substantially denature the protein. In addition, particles of substantially water insoluble pharmacologically active agents contained within a shell differ from crosslinked or heat denatured protein microspheres of the prior art because the polymeric shell produced by the invention process is relatively thin compared to the diameter of the coated particle. It has been determined (by transmission electron microscopy) that the "shell thickness" of the polymeric coat is approximately 25 nanometers for a coated particle having a diameter of 1 micron (1000 nanometers). In contrast, microspheres of the prior art do not have protein shells, but rather, have protein dispersed throughout the volume of the microsphere.

The polymeric shell containing solid or liquid cores of pharmacologically active agent allows for the delivery of high doses of the pharmacologically active agent in relatively small volumes. This minimizes patient discomfort at receiving large volumes of fluid and minimizes hospital stay. In addition, the walls of the polymeric shell are generally completely degradable in vivo by proteolytic enzymes (e.g., when the polymer is a protein), resulting in no side effects from the delivery system as is the case with current formulations.

According to this embodiment of the present invention, particles of substantially water insoluble pharmacologically active agents are contained within a shell having a cross-sectional diameter of no greater than about 10 microns. A cross-sectional diameter of less than 5 microns is more preferred, while a cross-sectional diameter of less than 1 micron is presently the most preferred for the intravenous route of administration.

Substantially water insoluble pharmacologically active agents contemplated for use in the practice of the present invention include pharmaceutically active agents, diagnostic agents, agents of nutritional value, and the like. Examples of pharmaceutically active agents include taxol (as used herein, the term "taxol" is intended to include taxol analogs and prodrugs, taxanes, and other taxol-like drugs, e.g., Taxotere, and the like), camptothecin and derivatives thereof (which compounds have great promise for the treatment of colon cancer), aspirin, ibuprofen, piroxicam, cimetidine, substantially water insoluble steroids (e.g., estrogen, prednisolone, cortisone, hydrocortisone, diflorasone, and the like), drugs such as phenesterine, duanorubicin, doxorubicin, mitotane, visadine, halonitrosoureas, anthrocylines, ellipticine, diazepam, and the like, anaesthetics such as methoxyfluorane, isofluorane, enfluorane, halothane, benzocaine, dantrolene, barbiturates, and the like. In addition, also contemplated are substantially water insoluble immunosuppressive agents, such as, for example, cyclosporines, azathioprine, FK506, prednisone, and the like. A presently preferred pharmaceutically active agent for use in the practice of the present invention is taxol, which is commercially available from the manufacturer as needle-like crystals.

Examples of diagnostic agents contemplated for use in the practice of the present invention include ultrasound contrast agents, radiocontrast agents (e.g., iodooctanes, halocarbons, renografin, and the like), magnetic contrast agents (e.g., fluorocarbons, lipid soluble paramagnetic compounds, and the like), as well as other diagnostic agents which cannot readily be delivered without some physical and/or chemical modification to accomodate the substantially water insoluble nature thereof.

Examples of agents of nutritional value contemplated for use in the practice of the present invention include amino acids, sugars, proteins, carbohydrates, fat-soluble vitamins (e.g., vitamins A, D, E, K, and the like) or fat, or combinations of any two or more thereof.

A number of biocompatible polymers may be employed in the practice of the present invention for the formation of the polymeric shell which surrounds the substantially water insoluble pharmacologically active agents. Essentially any polymer, natural or synthetic, bearing sulfhydryl groups or disulfide bonds within its structure may be utilized for the preparation of a disulfide crosslinked shell about particles of substantially water insoluble pharmacologically active agents. The sulfhydryl groups or disulfide linkages may be preexisting within the polymer structure or they may be introduced by a suitable chemical modification. For example, natural polymers such as proteins, oligopeptides, polynucleic acids, polysaccharides (e.g., starch, cellulose, dextrans, alginates, chitosan, pectin, hyaluronic acid, and the like), and so on, are candidates for such modification.

As examples of suitable biocompatible polymers, naturally occurring or synthetic proteins may be employed, so long as such proteins have sufficient cysteine residues within their amino acid sequences so that crosslinking (through disulfide bond formation, for example, as a result of oxidation during sonication) can occur. Examples of suitable proteins include albumin (which contains 35 cysteine residues), insulin (which contains 6 cysteines), hemoglobin (which contains 6 cysteine residues per $\alpha_2\beta_2$ unit), lysozyme (which contains 8 cysteine residues), immunoglobulins, α-2-macroglobulin, fibronectin, vitronectin, fibrinogen, and the like.

A presently preferred protein for use in the formation of a polymeric shell is albumin. Optionally, proteins such as α-2-macroglobulin, a known opsonin, could be used to enhance uptake of the shell encased particles of substantially water insoluble pharmacologically active agents by macrophage-like cells, or to enhance the uptake of the shell encased particles into the liver and spleen.

Similarly, synthetic polypeptides containing cysteine residues are also good candidates for formation of a shell about the substantially water insoluble pharmacologically active agents. In addition, polyvinyl alcohol, polyhydroxyethyl methacrylate, polyacrylic acid, polyethyloxazoline, polyacrylamide, polyvinyl pyrrolidinone, and the like, are good candidates for chemical modification (to introduce sulfhydryl and/or disulfide linkages) and shell formation (by causing the crosslinking thereof).

In the preparation of invention compositions, one can optionally employ a dispersing agent to suspend or dissolve the substantially water insoluble pharmacologically active agent. Dispersing agents contemplated for use in the practice of the present invention include any nonaqueous liquid that is capable of suspending or dissolving the pharmacologically active agent, but does not chemically react with either the polymer employed to produce the shell, or the pharmacologically active

agent itself. Examples include vegetable oils (e.g., soybean oil, coconut oil, olive oil, safflower oil, cotton seed oil, and the like), aliphatic, cycloaliphatic, or aromatic hydrocarbons having 4–30 carbon atoms (e.g., n-dodecane, n-decane, n-hexane, cyclohexane, toluene, benzene, and the like), aliphatic or aromatic alcohols having 2–30 carbon atoms (e.g., octanol, and the like), aliphatic or aromatic esters having 2–30 carbon atoms (e.g., ethyl caprylate (octanoate), and the like), alkyl, aryl, or cyclic ethers having 2–30 carbon atoms (e.g., diethyl ether, tetrahydrofuran, and the like), alkyl or aryl halides having 1–30 carbon atoms (and optionally more than one halogen substituent, e.g., CH_3Cl, CH_2Cl_2, $CH_2Cl—CH_2Cl$, and the like), ketones having 3–30 carbon atoms (e.g., acetone, methyl ethyl ketone, and the like), polyalkylene glycols (e.g., polyethylene glycol, and the like), or combinations of any two or more thereof.

Especially preferred combinations of dispersing agents include volatile liquids such as dichloromethane, ethyl acetate, benzene, and the like (i.e., solvents that have a high degree of solubility for the pharmacologically active agent, and are soluble in the other dispersing agent employed), along with a higher molecular weight (less volatile) dispersing agent. When added to the other dispersing agent, these volatile additives help to drive the solubility of the pharmacologically active agent into the dispersing agent. This is desirable since this step is usually time consuming. Following dissolution, the volatile component may be removed by evaporation (optionally under vacuum).

Particles of pharmacologically active agent substantially completely contained within a polymeric shell, prepared as described above, are delivered as a suspension in a biocompatible aqueous liquid. This liquid may be selected from water, saline, a solution containing appropriate buffers, a solution containing nutritional agents such as amino acids, sugars, proteins, carbohydrates, vitamins or fat, and the like.

In accordance with another embodiment of the present invention, there is provided a method for the preparation of a substantially water insoluble pharmacologically active agent for in vivo delivery, said method comprising subjecting a mixture comprising:

dispersing agent containing said pharmacologically active agent dispersed therein, and

aqueous medium containing biocompatible polymer capable of being crosslinked by disulfide bonds

to sonication conditions for a time sufficient to promote crosslinking of said biocompatible polymer by disulfide bonds.

A nonobvious feature of the above-described process is in the choice of dispersing agent, specifically with respect to the polarity of the dispersing agent. The formation of a shell about the particles of pharmacologically active agent involves unfolding and reorientation of the polymer at the interface between the aqueous and non-aqueous phases such that the hydrophilic regions within the polymer are exposed to the aqueous phase while the hydrophobic regions within the polymer are oriented towards the non-aqueous phase. In order to effect unfolding of the polymer, or change the conformation thereof, energy must be supplied to the polymer. The interfacial free energy (interfacial tension) between the two liquid phases (i.e., aqueous and non-aqueous) contributes to changes in polymer conformation at that interface. Thermal energy also contributes to the energy pool required for unfolding and/or change of polymer conformation.

Thermal energy input is a function of such variables as the acoustic power employed in the sonication process, the sonication time, the nature of the material being subjected to sonication, the volume of the material being subjected to sonication, and the like. The acoustic power of sonication processes can vary widely, typically falling in the range of about 1 up to 1000 watts/cm^2; with an acoustic power in the range of about 50 up to 200 watts/cm^2 being a presently preferred range. Similarly, sonication time can vary widely, typically falling in the range of a few seconds up to about 5 minutes. Preferably, sonication time will fall in the range of about 15 up to 60 seconds. Those of skill in the art recognize that the higher the acoustic power applied, the less sonication time is required, and vice versa.

The interfacial free energy is directly proportional to the polarity difference between the two liquids. Thus at a given operating temperature a minimum free energy at the interface between the two liquids is essential to form the desired polymer shell. Thus, if a homologous series of dispersing agents is taken with a gradual change in polarity, e.g., ethyl esters of alkanoic acids, then higher homologues are increasingly nonpolar, i.e., the interfacial tension between these dispersing agents and water increases as the number of carbon atoms in the ester increases. Thus it is found that, although ethyl acetate is water-immiscible (i.e., an ester of a 2 carbon acid), at room temperature (~20° C.), this dispersing agent alone will not give a significant yield of polymer shell-coated particles. In contrast, a higher ester such as ethyl octanoate (ester of an 8 carbon acid) gives polymer shell-coated particles in high yield. In fact, ethyl heptanoate (ester of a 7 carbon acid) gives a moderate yield while the lower esters (esters of 3, 4, 5, or 6 carbon acids) give poor yield. Thus, at a given temperature, one could set a condition of minimum aqueous-dispersing agent interfacial tension required for formation of high yields of polymer shell-coated particles.

Temperature is another variable that may be manipulated to affect the yield of polymer shell-coated particles. In general the surface tension of a liquid decreases with increasing temperature. The rate of change of surface tension with temperature is often different for different liquids. Thus, for example, the interfacial tension ($\Delta\gamma$) between two liquids may be $\Delta\gamma_1$ at temperature T_1 and $\Delta\gamma_2$ at temperature T_2. If $\Delta\gamma_1$ at T_1 is close to the minimum required to form polymeric shells of the present invention, and if $\Delta\gamma_2$ (at temp. T_2) is greater than $\Delta\gamma_1$, then a change of temperature from T_1 to T_2 will increase the yield of polymeric shells. This, in fact, is observed in the case of ethyl heptanoate, which gives a moderate yield at 20° C. but gives a high yield at 10° C.

Temperature also affects the vapor pressure of the liquids employed. The lower the temperature, the lower the total vapor pressure. The lower the total vapor pressure, the more efficient is the collapse of the cavitation bubble. A more efficient collapse of the sonication bubble correlates with an increased rate of superoxide (HO_2^-) formation. Increased rate of superoxide formation leads to increased yields of polymeric shells at lower temperatures. As a countervailing consideration, however, the reaction rate for oxidation of sulfhydryl groups (i.e., to form disulfide linkages) by superoxide ions increases with increasing temperature. Thus for a

given liquid subjected to sonication conditions, there exists a fairly narrow range of optimum operating temperatures within which a high yield of polymeric shells is obtained.

Thus a combination of two effects, i.e., the change in surface tension with temperature (which directly affects unfolding and/or conformational changes of the polymer) and the change in reaction yield (the reaction being crosslinking of the polymer via formation of disulfide linkages) with temperature dictate the overall conversion or yield of polymer shell-coated particles.

The sonication process described above may be manipulated to produce polymer shell-coated particles containing pharmacologically active agent having a range of sizes. Presently preferred particle radii fall in the range of about 0.1 up to about 5 micron. A narrow size distribution in this range is very suitable for intravenous drug delivery. The polymer shell-coated particles are then suspended in an aqueous biocompatible liquid (as described above) prior to administration by suitable means.

Variations on the general theme of dissolved pharmacologically active agent enclosed within a polymeric shell are possible. A suspension of fine particles of pharmacologically active agent in a biocompatible dispersing agent could be used (in place of a biocompatible dispersing agent containing dissolved pharmacologically active agent) to produce a polymeric shell containing dispersing agent-suspended pharmacologically active agent particles. In other words, the polymeric shell could contain a saturated solution of pharmacologically active agent in dispersing agent. Another variation is a polymeric shell containing a solid core of pharmacologically active agent produced by initially dissolving the pharmacologically active agent in a volatile organic solvent (e.g. benzene), forming the polymeric shell and evaporating the volatile solvent under vacuum, e.g., in a rotary evaporator, or freeze-drying the entire suspension. This results in a structure having a solid core of pharmacologically active agent surrounded by a polymer coat. This latter method is particularly advantageous for delivering high doses of pharmacologically active agent in a relatively small volume. In some cases, the polymer forming the shell about the core could itself be a therapeutic or diagnostic agent, e.g., in the case of insulin, which may be delivered as part of a polymeric shell formed in the sonication process described above.

Variations in the polymeric shell are also possible. For example, a small amount of PEG containing sulfhydryl groups could be included with the polymer. Upon sonication, the PEG is crosslinked into the polymer and forms a component of the polymeric shell. PEG is known for its nonadhesive character and has been attached to proteins and enzymes to increase their circulation time in vivo [Abuchowski et al., J. Biol. Chem. Vol. 252:3578 (1977)]. It has also been attached to phospholipids forming the lipidic bilayer in liposomes to reduce their uptake and prolong lifetimes in vivo [Klibanov et al., FEBS Letters Vol. 268:235 (1990)]. Thus the incorporation of PEG into the walls of crosslinked protein shells alters their blood circulation time. This property can be exploited to maintain higher blood levels of the pharmacologically active agent and prolonged pharmacologically active agent release times.

One skilled in the art will recognize that several variations are possible within the scope and spirit of this invention. The dispersing agent within the polymeric shell may be varied, a large variety of pharmacologically active agents may be utilized, and a wide range of proteins as well as other natural and synthetic polymers may be used in the formation of the walls of the polymeric shell. Applications are also fairly wide ranging. Other than biomedical applications such as the delivery of drugs, diagnostic agents (in imaging applications), artificial blood (sonochemically crosslinked hemoglobin) and parenteral nutritional agents, the polymeric shell structures of the invention may be incorporated into cosmetic applications such as skin creams or hair care products, in perfumery applications, in pressure sensitive inks, and the like.

An approach to the problem of taxol administration that has not been described in the literature is its delivery as an aqueous suspension of micron size particles, or an aqueous suspension containing either particles of taxol or taxol dissolved in a biocompatible non-aqueous liquid. This approach would facilitate the delivery of taxol at relatively high concentrations and obviate the use of emulsifiers and their associated toxic side effects.

In accordance with yet another embodiment of the present invention, the above-described mode of administration is facilitated by novel taxol-containing compositions wherein taxol is suspended in a biocompatible liquid, and wherein the resulting suspension contains particles of taxol having a cross-sectional dimension no greater than about 10 microns. The desired particle size of less than about 10 microns can be achieved in a variety of ways, e.g., by grinding, spray drying, precipitation, sonication, and the like.

Due to the crystal size of conventionally obtained taxol which is greater than 20 microns, solid particles of taxol have not been delivered in the form of a suspension in a vehicle such as normal saline. However, the present invention discloses the delivery of a particulate suspension of taxol ground to a size less than 10 microns, preferably less than 5 microns and most preferably less than 1 micron, which allows intravenous delivery in the form of a suspension without the risk of blockage in the microcirculation of organs and tissues.

Due to the microparticular nature of the delivered drug, most of it is cleared from the circulation by organs having reticuloendothelial systems such as the spleen, liver, and lungs. This allows pharmacologically active agents in particulate form to be targeted to such sites within the body.

Biocompatible liquids contemplated for use in this embodiment are the same as those described above. In addition, parenteral nutritional agents such as Intralipid (trade name for a commercially available fat emulsion used as a parenteral nutrition agent; available from Kabi Vitrum, Inc., Clayton, N.C.), Nutralipid (trade name for a commercially available fat emulsion used as a parenteral nutrition agent; available from McGaw, Irvine, Calif.), Liposyn III (trade name for a commercially available fat emulsion used as a parenteral nutrition agent (containing 20% soybean oil, 1.2% egg phosphatides, and 2.5% glycerin); available from Abbott Laboratories, North Chicago, Ill.), and the like may be used as the carrier of the drug particles. Alternatively, if the biocompatible liquid contains a drug-solubilizing material such as soybean oil (e.g., as in the case of Intralipid), the drug may be partially or completely solubilized within the carrier liquid, aiding its delivery. An example of such a case is the delivery of taxol in Intralipid as the carrier. Presently preferred biocompati-

ble liquids for use in this embodiment are parenteral nutrition agents, such as those described above.

In accordance with still another embodiment of the present invention, there is provided a composition for the in vivo delivery of taxol wherein taxol is dissolved in a parenteral nutrition agent.

The invention will now be described in greater detail by reference to the following non-limiting examples.

EXAMPLE 1

Preparation of Taxol Particles

Crystals of taxol (Sigma Chemical) were ground in a ball mill until particles of solid taxol were obtained having a size less than 10 microns. Size of particles were determined by suspending the particles in isotonic saline and counting with the aid of a particle counter (Elzone, Particle Data). Grinding was continued until 100% of the particles had a size less than 5 microns. The preferred particle size for intravenous delivery is less than 5 microns and most preferably less than 1 micron.

Alternatively, particles of taxol were obtained by sonicating a suspension of taxol in water until all particles were below 10 microns.

Taxol particles less than 10 microns can also be obtained by precipitating taxol from a solution of taxol in ethanol by adding water until a cloudy suspension is obtained. Optionally, the solution of taxol can be sonicated during the water addition, until a cloudy suspension is obtained. The resulting suspension is then filtered and dried to obtain pure taxol particles in the desired size range.

Fine particles of taxol were prepared by spray drying a solution of taxol in a volatile organic such as ethanol. The solution was passed through an ultrasonic nozzle that formed droplets of ethanol containing taxol. As the ethanol evaporated in the spray drier, fine particles of taxol were obtained. Particle size can be varied by changing the concentration of taxol in ethanol, adjusting the flow rate of liquid through the nozzle and power of sonication.

EXAMPLE 2

Preparation of Protein Shell Containing Oil

Three ml of a USP (United States Pharmacop ia) 5% human serum albumin solution (Alpha Therapeutic Corporation) were taken in a cylindrical vessel that could be attached to a sonicating probe (Heat Systems, Model XL2020). The albumin solution was overlayered with 6.5 ml of USP grade soybean oil (soya oil). The tip of the sonicator probe was brought to the interface between the two solutions and the assembly was maintained in a cooling bath at 20° C. The system was allowed to equilibriate and the sonicator turned on for 30 seconds. Vigorous mixing occurred and a white milky suspension was obtained. The suspension was diluted 1:5 with normal saline. A particle counter (Particle Data Systems, Elzone, Model 280 PC) was utilized to determine size distribution and concentration of oil-containing protein shells. The resulting protein shells were determined to have a maximum cross-sectional dimension of about 1.35 ± 0.73 microns, and the total concentration determined to be $\sim 10^9$ shells/ml in the original suspension.

EXAMPLE 3

Parameters Affecting Polymeric Shell Formation

Several variables such as protein concentration, temperature, sonication time, concentration of pharmacologically active agent, and acoustic intensity were tested to optimize formation of polymeric shell. These parameters were determined for crosslinked bovine serum albumin shells containing toluene.

Polymeric shells made from solutions having protein concentrations of 1%, 2.5%, 5% and 10% were counted with the particle counter to determine a change in the size and number of polymeric shells produced. The size of the polymeric shells was found not to vary with protein concentration, but the number of polymeric shells per ml of "milky suspension" formed increased with the increase in concentration of the protein up to 5%. No significant change in the number of polymeric shells was found to occur above that concentration.

Initial vessel temperatures were found to be important for optimal preparation of polymeric shells. Typically, initial vessel temperatures were maintained between 0° C. and 45° C. The aqueous-oil interfacial tension of the oils used for formation of the polymeric shell was an important parameter, which also varied as a function of temperature. The concentration of pharmacologically active agent was found not to significantly effect the yield of protein shells. It is relatively unimportant if the pharmacologically active agent is incorporated in the dissolved state, or suspended in the dispersing medium.

Sonication time was an important factor determining the number of polymeric shells produced per ml. It was found that a sonication time greater than three minutes produced a decrease in the overall count of polymeric shells, indicating possible destruction of polymeric shells due to excessive sonication. Sonication times less than three minutes were found to produce adequate numbers of polymeric shells.

According to the nomograph provided by the manufacturer of the sonicator, the acoustic power rating of the sonicator employed herein is approximately 150 watts/cm^2. Three power settings in order of increasing power were used, and it was found that the maximum number of polymeric shells were produced at the highest power setting.

EXAMPLE 4

Preparation of Polymeric Shells Containing Dissolved Taxol

Taxol was dissolved in USP grade soybean oil at a concentration of 2 mg/ml. 3 ml of a USP 5% human serum albumin solution was taken in a cylindrical vessel that could be attached to a sonicating probe. The albumin solution was overlayered with 6.5 ml of soybean oil/taxol solution. The tip of the sonicator probe was brought to the interface between the two solutions and the assembly was maintained in equilibrium and the sonicator turned on for 30 seconds. Vigorous mixing occurred and a stable white milky suspension was obtained which contained protein-walled polymeric shells enclosing the oil/taxol solution.

In order to obtain a higher loading of drug into the crosslinked protein shell, a mutual solvent for the oil and the drug (in which the drug has a considerably higher solubility) can be mixed with the oil. Provided

this solvent is relatively non-toxic (e.g., ethyl acetate), it may be injected along with the original carrier. In other cases, it may be removed by evaporation of the liquid under vacuum following preparation of the polymeric shells.

EXAMPLE 5

Stability of Polymeric Shells

Suspensions of polymeric shells at a known concentration were analyzed for stability at three different temperatures (i.e., 4° C., 25° C., and 38° C.). Stability was measured by the change in particle counts over time. Crosslinked protein (albumin) shells containing soybean oil (SBO) were prepared as described above (see Example 2), diluted in saline to a final oil concentration of 20% and stored at the above temperatures. Particle counts (Elzone) obtained for each of the samples as a function of time are summarized in Table 1.

TABLE 1

	Protein Shells (#/ml · 10^{10}) in saline		
Day	4° C.	25° C.	38° C.
0	7.9	8.9	8.1
1	7.4	6.9	6.8
7	7.3	8.3	5.0
9	7.8	8.1	5.8
17	7.8	8.3	6.1
23	6.9	7.8	7.4
27	7.2	8.8	7.1

As demonstrated by the above data, the concentration of counted particles (i.e., polymeric shells) remains fairly constant over the duration of the experiment. The range is fairly constant and remains between about 7-9·10^{10}/ml, indicating good polymeric shell stability under a variety of temperature conditions over almost four weeks.

EXAMPLE 6

In Vivo Biodistribution—Crosslinked Protein Shells Containing a Fluorophore

To determine the fate of crosslinked albumin shells following intravenous injection, a fluorescent dye (rubrene, obtained from Aldrich) was dissolved in toluene, and crosslinked albumin shells containing toluene/rubrene were prepared as described above by sonication. The resulting milky suspension was diluted five times in normal saline. Two ml of the diluted suspension was then injected into the tail vein of a rat over 10 minutes. One animal was sacrificed an hour after injection and another 24 hours after injection.

Frozen lung, liver, kidney, spleen, and bone marrow sections were examined under fluorescence for the presence of polymeric shells containing fluorescent dye. At one hour, most of the polymeric shells were intact and found in the lungs and liver as brightly fluorescing particles of about 1 micron diameter. At 24 hours, polymeric shells were found in the liver, lungs, spleen, and bone marrow. A general staining of the tissue was also observed, indicating that the polymeric shells had been digested, and the dye liberated from within. This result was consistent with expectations and demonstrates the potential use of invention compositions for delayed or controlled release of entrapped pharmaceutical agent such as taxol.

EXAMPLE 7

Toxicity of Polymeric Shells Containing Soybean Oil (SBO)

Polymeric shells containing soybean oil were prepared as described in Example 2. The resulting suspension was diluted in normal saline to produce two different solutions, one containing 20% SBO and the other containing 30% SBO.

Intralipid, a commercially available TPN agent, contains 20% SBO. The LD_{50} for Intralipid in mice is 120 ml/kg, or about 4 ml for a 30 g mouse, when injected at 1 cc/min.

Two groups of mice (three mice in each group; each mouse weighing about 30 g) were treated with invention composition containing SBO as follows. Each mouse was injected with 4 ml of the prepared suspension of SBO-containing polymeric shells. Each member of one group received the suspension containing 20% SBO, while each member of the other group received the suspension containing 30% SBO.

All three mice in the group receiving the suspension containing 20% SBO survived such treatment, and showed no gross toxicity in any tissues or organs when observed one week after SBO treatment. Only one of the three mice in the group receiving suspension containing 30% SBO died after injection. These results clearly demonstrate that oil contained within polymeric shells according to the present invention is not toxic at its LD_{50} dose, as compared to a commercially available SBO formulation (Intralipid). This effect can be attributed to the slow release (i.e., controlled rate of becoming bioavailable) of the oil from within the polymeric shell. Such slow release prevents the attainment of a lethal dose of oil, in contrast to the high oil dosages attained with commercially available emulsions.

EXAMPLE 8

In vivo Bioavailability of Soybean Oil Released from Polymeric Shells

A test was performed to determine the slow or sustained release of polymeric shell-enclosed material following the injection of a suspension of polymeric shells into the blood stream of rats. Crosslinked protein (albumin) walled polymeric shells containing soybean oil (SBO) were prepared by sonication as described above. The resulting suspension of oil-containing polymeric shells was diluted in saline to a final suspension containing 20% oil. Five ml of this suspension was injected into the cannulated external jugular vein of rats over a 10 minute period. Blood was collected from these rats at several time points following the injection and the level of triglycerides (soybean oil is predominantly triglyceride) in the blood determined by routine analysis.

Five ml of a commercially available fat emulsion (Intralipid, an aqueous parenteral nutrition agent—containing 20% soybean oil, 1.2% egg yolk phospholipids, and 2.25% glycerin) was used as a control. The control utilizes egg phosphatide as an emulsifier to stabilize the emulsion. A comparison of serum levels of the triglycerides in the two cases would give a direct comparison of the bioavailability of the oil as a function of time. In addition to the suspension of polymeric shells containing 20% oil, five ml of a sample of oil-containing polymeric shells in saline at a final concentration of 30% oil was also injected. Two rats were used in each of the

three groups. The blood levels of triglycerides in each case are tabulated in Table 2, given in units of mg/dl.

TABLE 2

GROUP	SERUM TRIGLYCERIDES (mg/dl) Pre	1 hr	4 hr	24 hr	48 hr	72 hr
Intralipid Control (20% SBO)	11.4	941.9	382.9	15.0	8.8	23.8
Polymeric Shells (20% SBO)	24.8	46.7	43.8	29.3	24.2	43.4
Polymeric Shells (30% SBO)	33.4	56.1	134.5	83.2	34.3	33.9

Blood levels before injection are shown in the column marked 'Pre'. Clearly, for the Intralipid control, very high triglyceride levels are seen following injection. Triglyceride levels are then seen to take about 24 hours to come down to preinjection levels. Thus the oil is seen to be immediately available for metabolism following injection.

The suspension of oil-containing polymeric shells containing the same amount of total oil as Intralipid (20%) show a dramatically different availability of detectible triglyceride in the serum. The level rises to about twice its normal value and is maintained at this level for many hours, indicating a slow or sustained release of triglyceride into the blood at levels fairly close to normal. The group receiving oil-containing polymeric shells having 30% oil shows a higher level of triglycerides (concomitant with the higher administered dose) that falls to normal within 48 hours. Once again, the blood levels of triglyceride do not rise astronomically in this group, compared to the control group receiving Intralipid. This again, indicates the slow and sustained availability of the oil from invention composition, which has the advantages of avoiding dangerously high blood levels of material contained within the polymeric shells and availability over an extended period at acceptable levels. Clearly, drugs delivered within polymeric shells of the present invention would achieve these same advantages.

Such a system of soybean oil-containing polymeric shells could be suspended in an aqueous solution of amino acids, essential electrolytes, vitamins, and sugars to form a total parenteral nutrition (TPN) agent. Such a TPN cannot be formulated from currently available fat emulsions (e.g., Intralipid) due to the instability of the emulsion in the presence of electrolytes.

EXAMPLE 9

Preparation of Crosslinked Protein-walled Polymeric Shells Containing a Solid Core of Pharmaceutically Active Agent

Another method of delivering a poorly water-soluble drug such as taxol within a polymeric shell is to prepare a shell of polymeric material around a solid drug core. Such a 'protein coated' drug particle may be obtained as follows. The procedure described in Example 4 is repeated using an organic solvent to dissolve taxol at a relatively high concentration. Solvents generally used are organics such as benzene, toluene, hexane, ethyl ether, and the like. Polymeric shells are produced as described in Example 4. Five ml of the milky suspension of polymeric shells containing dissolved taxol are diluted to 10 ml in normal saline. This suspension is placed in a rotary evaporator at room temperature and the volatile organic removed by vacuum. After about 2 hours in the rotary evaporator, these polymeric shells are examined under a microscope to reveal opaque cores, indicating removal of substantially all organic solvent, and the presence of solid taxol within a shell of protein.

Alternatively, the polymeric shells with cores of organic solvent-containing dissolved drug are freeze-dried to obtain a dry crumbly powder that can be resuspended in saline (or other suitable liquid) at the time of use. In case of other drugs that may not be in the solid phase at room temperature, a liquid core polymeric shell is obtained. This method allows for the preparation of a crosslinked protein-walled shell containing undiluted drug within it. Particle size analysis shows these polymeric shells to be smaller than those containing oil. Although the presently preferred protein for use in the formation of the polymeric shell is albumin, other proteins such as α-2-macroglobulin, a known opsonin, could be used to enhance uptake of the polymeric shells by macrophage-like cells. Alternatively, a PEG-sulfhydryl (described below) could be added during formation of the polymeric shell to produce a polymeric shell with increased circulation time in vivo.

EXAMPLE 10

In vivo Circulation and Release Kinetics of Polymeric Shells

Solid core polymeric shells containing taxol were prepared as described above (see, for example, Example 4) and suspended in normal saline. The concentration of taxol in the suspension was measured by HPLC as follows. First, the taxol within the polymeric shell was liberated by the addition of 0.1M mercaptoethanol (resulting in exchange of protein disulfide crosslinkages, and breakdown of the crosslinking of the polymeric shell), then the liberated taxol was extracted from the suspension with acetonitrile. The resulting mixture was centrifuged and the supernatant freeze-dried. The lyophilate was dissolved in methanol and injected onto an HPLC to determine the concentration of taxol in the suspension. The taxol concentration was found to be about 1.6 mg/ml.

Rats were injected with 2 ml of this suspension through a jugular catheter. The animal was sacrificed at two hours, and the amount of taxol present in the liver determined by HPLC. This required homogenization of the liver, followed by extraction with acetonitrile and lyophilization of the supernatant following centrifugation. The lyophilate was dissolved in methanol and injected onto an HPLC. Approximately 15% of the administered dose of taxol was recovered from the liver at two hours, indicating a significant dosage to the liver. This result is consistent with the known function of the reticuloendothelial system of the liver in clearing small particles from the blood.

EXAMPLE 11

Preparation of Crosslinked PEG-walled Polymeric Shells

As an alternative to the use of thiol (sulfhydryl) containing proteins in the formation of, or as an additive to polymeric shells of the invention, a thiol-containing PEG was prepared. PEG is known to be nontoxic, noninflammatory, nonadhesive to cells, and in general biologically inert. It has been bound to proteins to reduce their antigenicity and to liposome forming lipids to increase their circulation time in vivo. Thus incorporation of PEG into an essentially protein shell would be

expected to increase circulation time as well as stability of the polymeric shell. By varying the concentration of PEG-thiol added to the 5% albumin solution, it was possible to obtain polymeric shells with varying stabilities in vivo. PEG-thiol was prepared by techniques available in the literature (such as the technique of Harris and Herati, as described in Polymer Preprints Vol. 32:154–155 (1991)).

PEG-thiol of molecular weight 2000 g/mol was dissolved at a concentration of 1% (0.1 g added to 10 ml) in a 5% albumin solution. This protein/PEG solution was overlayered with oil as described in Example 2 and sonicated to produce oil-containing polymeric shells with walls comprising crosslinked protein and PEG. These Polymeric shells were tested for stability as described in Example 5.

Other synthetic water-soluble polymers that may be modified with thiol groups and utilized in lieu of PEG include, for example, polyvinyl alcohol, polyhydroxyethyl methacrylate, polyacrylic acid, polyethyloxazoline, polyacrylamide, polyvinyl pyrrolidinone, polysaccharides (such as chitosan, alginates, hyaluronic acid, dextrans, starch, pectin, etc), and the like.

EXAMPLE 12

Targeting of Immunosuppressive Agent to Transplanted Organs using Intravenous Delivery of Polymeric Shells Containing Such Agents

Immunosuppressive agents are extensively used following organ transplantation for the prevention of rejection episodes. In particular, cyclosporine, a potent immunosuppressive agent, prolongs the survival of allogeneic transplants involving skin, heart, kidney, pancreas, bone marrow, small intestine, and lung in animals. Cyclosporine has been demonstrated to suppress some humoral immunity and to a greater extent, cell mediated reactions such as allograft rejection, delayed hypersensitivity, experimental allergic encephalomyelitis, Freund's adjuvant arthritis, and graft versus host disease in many animal species for a variety of organs. Successful kidney, liver and heart allogeneic transplants have been performed in humans using cyclosporine.

Cyclosporine is currently delivered in oral form either as capsules containing a solution of cyclosporine in alcohol, and oils such as corn oil, polyoxyethylated glycerides and the like, or as a solution in olive oil, polyoxyethylated glycerides, etc. It is also administered by intravenous injection, in which case it is dissolved in a solution of ethanol (approximately 30%) and Cremaphor (polyoxyethylated castor oil) which must be diluted 1:20 to 1:100 in normal saline or 5% dextrose prior to injection. Compared to an intravenous (i.v.) infusion, the absolute bioavailibility of the oral solution is approximately 30% (Sandoz Pharmaceutical Corporation, Publication SDI-Z10 (A4), 1990). In general, the i.v. delivery of cyclosporine suffers from similar problems as the currently practiced i.v. delivery of taxol, i.e., anaphylactic and allergic reactions believed to be due to the Cremaphor, the delivery vehicle employed for the i.v. formulation.

In order to avoid problems associated with the Cremaphor, cyclosporine contained within polymeric shells as described above may be delivered by i.v. injection. It may be dissolved in a biocompatible oil or a number of other solvents following which it may be dispersed into polymeric shells by sonication as described above. In addition, an important advantage to delivering cyclosporine (or other immunosuppressive agent) in polymeric shells has the advantage of local targeting due to uptake of the injected material by the RES system in the liver. This may, to some extent, avoid systemic toxicity and reduce effective dosages due to local targeting. The effectiveness of delivery and targeting to the liver of taxol contained within polymeric shells following intravenous injection is demonstrated in Example 9. A similar result would be expected for the delivery of cyclosporine (or other putative immunosuppressive agent) in accordance with the present invention.

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 composition for in vivo delivery of a substantially water insoluble pharmacologically active agent,
   wherein said agent is a solid or liquid substantially completely contained within a polymeric shell,
   wherein the largest cross-sectional dimension of said shell is no greater than about 10 microns,
   wherein said polymeric shell comprises a biocompatible polymer which is substantially crosslinked by way of disulfide bonds, and
   wherein said polymeric shell containing pharmacologically active agent therein is suspended in a biocompatible aqueous liquid.

2. A composition 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.

3. A composition according to claim 2 wherein said pharmaceutically active agent is selected from taxol, taxotere, campothecin, aspirin, ibuprofen, piroxicam, cimetidine, substantially water insoluble steroids, phenesterine, duanorubicin, doxorubicin, mitotane, visadine, halonitrosoureas, anthrocylines, ellipticine, diazepam, methoxyfluorane, isofluorane, enfluorane, halothane, benzocaine, dantrolene, or barbiturates.

4. A composition according to claim 2 wherein said diagnostic agent is selected from ultrasound contrast agents, radiocontrast agents, or magnetic contrast agents.

5. A composition according to claim 2 wherein said agent of nutritional value is selected from amino acids, sugars, proteins, carbohydrates, fat-soluble vitamins, fat, or combinations of any two or more thereof.

6. A composition according to claim 2 wherein said pharmacologically active agent within said shell is dissolved in a biocompatible dispersing agent.

7. A composition according to claim 6 wherein said biocompatible dispersing agent is selected from soybean oil, coconut oil, olive oil, safflower oil, cotton seed 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 substutuent, ketones having 3–30 carbon atoms, polyalkylene glycol, or combinations of any two or more thereof.

8. A composition according to claim 2 wherein said pharmacologically active agent within said shell is suspended in a biocompatible dispersing agent.

9. A composition according to claim 8 wherein said biocompatible dispersing agent is selected from soybean oil, coconut oil, olive oil, safflower oil, cotton seed 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 substutuent, ketones having 3–30 carbon atoms, polyalkylene glycol, or combinations of any two or more thereof.

10. A composition according to claim 2 wherein said pharmacologically active agent within said shell is not diluted.

11. A composition according to claim 1 wherein said crosslinked polymer is a naturally occurring polymer, a synthetic polymer, or a combination thereof,

wherein said polymer, prior to crosslinking, has covalently attached thereto sulfhydryl groups or disulfide linkages.

12. A composition according to claim 11 wherein said naturally occurring polymers are selected from proteins, lipids, polynucleic acids or polysaccharides.

13. A composition according to claim 11 wherein said synthetic polymers are selected from synthetic polyamino acids containing cysteine residues and/or disulfide groups, 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, as well as mixtures of any two or more thereof.

14. A composition according to claim 1 wherein the disulfide bonds on the crosslinked polymer are formed by sonication.

15. A composition according to claim 1 wherein said biocompatible polymer is the crosslinked protein albumin.

16. A composition according to claim 1 wherein said biocompatible aqueous liquid 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.

17. A method for the delivery of substantially water insoluble pharmaceutical agents to a subject, said method comprising administering to said subject an effective amount of composition according to claim 1.

* * * * *

EXHIBIT -2

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United States Patent [19]

Desai et al.

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[45] Date of Patent: Aug. 1, 2000

[54] METHODS AND COMPOSITIONS USEFUL FOR ADMINISTRATION OF CHEMOTHERAPEUTIC AGENTS

[75] Inventors: Neil P. Desai; Patrick Soon-Shiong, both of Los Angeles, Calif.

[73] Assignee: Vivorx Pharmaceuticals, Inc., Santa Monica, Calif.

[21] Appl. No.: 08/926,155

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Related U.S. Application Data

[63] Continuation-in-part of application No. 08/720,756, Oct. 1, 1996, Pat. No. 5,916,596, which is a continuation-in-part of application No. 08/485,448, Jun. 7, 1995, Pat. No. 5,665,382, which is a continuation-in-part of application No. 08/200,235, Feb. 22, 1994, Pat. No. 5,498,421, which is a continuation-in-part of application No. 08/023,698, Feb. 22, 1993, Pat. No. 5,439,686, and a continuation-in-part of application No. 08/035,150, Mar. 26, 1993, Pat. No. 5,362,478.

[51] Int. Cl.[7] A61K 9/127

[52] U.S. Cl. 424/422; 424/489; 424/426; 424/455; 424/428

[58] Field of Search 424/450, 422, 424/489, 426, 491, 497; 514/44, 359, 358

[56] References Cited

U.S. PATENT DOCUMENTS

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5,626,862	5/1997	Brem et al.	424/426
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Primary Examiner—Thurman K. Page
Assistant Examiner—William E. 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 a pharmaceutically active agent, wherein the agent is associated with a polymeric biocompatible material.

57 Claims, No Drawings

METHODS AND COMPOSITIONS USEFUL FOR ADMINISTRATION OF CHEMOTHERAPEUTIC AGENTS

RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Ser. No. 08/720,756, filed Oct. 1, 1996, now issued as U.S. Pat. No. 5,916,596, and U.S. Ser. No.08/485,448, filed Jun. 7, 1995, now U.S. Pat. No. 5,665,382, which is, in turn, a continuation-in-part of U.S. Ser. No. 08/200,235, filed Feb. 22, 1994, now issued as U.S. Pat. No. 5,498,421, which is, in turn, a continuation-in-part of U.S. Ser. No. 08/023,698, filed Feb. 22, 1993, now issued as U.S. Pat. No. 5,439,626, and U.S. Ser. No. 08/035,150, filed Mar. 26, 1993, now issued as U.S. Pat. No. 5,362,478, the contents of each of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention relates to in vivo delivery of biologics such as the anticancer drug paclitaxel. The invention relates to the method of use and preparation of compositions (formulations) of drugs such as the anticancer agent paclitaxel. In one aspect, the formulation of paclitaxel, known as Capxol, has been found to be significantly less toxic and more efficacious than TAXOL, a commercially available formulation of paclitaxel. In another aspect, the novel formulation Capxol, has been found to localize in certain tissues after parenteral administration, thereby increasing the efficacy of treatment of cancers associated with such tissues.

BACKGROUND OF THE INVENTION

Taxol is a naturally occurring compound which has shown great promise as an anti-cancer drug. For example taxol has been found to be an active agent against drug-refractory ovarian cancer by McGuire et al. See "Taxol: A unique Anti-Neoplastic Agent With Significant Activity Against Advanced Ovarian Epithelial Neoplasms." Ann. Int. Med., 111, 273–279 (1989). All patents, scientific articles, and other documents mentioned herein are incorporated by reference as if reproduced in full below.

Unfortunately, taxol has extremely low solubility in water, which makes it difficult to provide a suitable dosage form. In fact, in Phase I clinical trials, severe allergic reactions were caused by the emulsifiers administered in conjunction with taxol to compensate for taxol's low water solubility; at least one patient's death was caused by an allergic reaction induced by the emulsifiers. Dose limiting toxicities include neutropenia, peripheral neuropathy, and hypersensitivity reactions (HSRs).

Brown et al., in "A Phase I Trial of Taxol Given by A 6-Hour Intravenous Infusion" J of Clin Oncol, Vol. 9 No. 7, pp. 1261–1267 (July 1991) report on a Phase I Trial in which taxol was provided as a 6 -hour IV infusion every 21 days without premedication. 31 patients received 64 assessable courses of taxol. One patient had a severe (or acute) hypersensitivity reaction, which required discontinuation of the infusion and immediate treatment to save the patient's life. Another patient experienced a hypersensitivity reaction, but it was not so sever as to require discontinuing the infusion. Myelosuppression was dose-limiting, with 2 fatalities due to sepsis. Non-hematologic toxicity was of Grade 1 and 2, except for one patient with Grade 3 mucositis and 2 patients with Grade 3 neuropathy. The neuropathy consisted of reversible painful paresthesias, requiring discontinuation of taxol in two patients. Four partial responses were seen (3 in patients with non-small-cell lung cancer, and one in a patient with adenocarcinoma of unknown primary). The maximum tolerated dose reported was 275 mg/m^2, and the recommended Phase II starting dose was 225 mg/m^2. The incidence of hypersensitivity reaction was reported to be schedule-dependent, with 6 to 24-hour infusions of drug having a 0% to 8% incidence of hypersensitivity reactions. It was also reported that hypersensitivity reactions persist with or without premedication, despite prolongation of infusion times. Since these Phase I studies were conducted on terminally ill patients suffering from a variety of cancer, the efficacy of the taxol treatments could not be determined.

In a study by Kris et al, taxol formulated with Cremaphor EL in dehydrated alcohol was given as a 3-hour IV infusion every 21 days, with the administered dosage ranging from 15 to 230 mg/m^2 in nine escalation steps. Kris et al. concluded that "with the severity and unpredictability of the hypersensitivity reactions, further usage of taxol is not indicated with this drug formulation on this administration schedule." See Cancer Treat. Rep., Vol. 70, No. 5, May 1986.

Since early trials using a bolus injection or short (1–3 hour) infusions induced anaphylactic reactions or other hypersensitivity responses, further studies were carried out in which taxol was administered only after premedication with steroids (such as dexamethasone), antihistamines (such as diphenhydramine), and H2-antagonists (such as cimetidine or ranitidine), and the infusion time was extended to 24 hours in an attempt to eliminate the most serious allergic reactions. Various Phase I and Phase II study results have been published utilizing 24-hour infusions of taxol with maximum total dosages of 250 mg/m2, generally with the course being repeated every 3 weeks. Patients were pretreated with dexamethasone, diphenhydramine, and cimetidine to offset allergic reactions. See Einzig, et al., "Phase II Trial of Taxol in Patients with Metastatic Renal Cell Carcinoma," Cancer Investigation, 9(2) 133–136 (1991), and A. B. Miller et al., "Reporting Results of Cancer Treatment," Cancer, Vol 47, 207–214 (1981).

Koeller et al., in "A Phase I Pharmacokinetic Study of Taxol Given By a Prolonged Infusion Without Premedication," Proceedings of ASCO, Vol. 8 (March, 1989), recommends routine premedication in order to avoid the significant number of allergic reactions believed to be caused by the cremophor (polyethyoxylated castor oil) vehicle used for taxol infusion. Patients received dosages ranging from 175 mg/m^2 to 275 mg/m^2.

Wiernik et al. in "Phase I Clinical and Pharmacokinetic Study of Taxol," Cancer Research, 47, 2486–2493 (May 1, 1987), also report the administration of taxol in a cremophor vehicle by IV infusion over a 6-hour period in a Phase I study. Grade 3–4 hypersensitivity reactions incurred in 4 of 13 courses. The starting dose for the study was 15 mg/m^2 (one-third of the lowest toxic dose in dogs). Doses were escalated, and a minimum of 3 patients were treated at each dose level until toxicity was identified, and then 4–6 patients were treated at each subsequent level. The study concluded that neurotoxicity and leukopenia were dose-limiting, and the recommended Phase II trial dose was 250 mg/m^2 with premedication.

Other exemplary studies on taxol include: Legha et al., "Phase II Trail of Taxol in Metastatic Melanoma," Vol. 65 (June 1990) pp. 2478–2481; Rowinsky et al., "Phase I and Pharmacodynamic Study of Taxol in Refractory Acute Leukemias," Cancer Research, 49, 4640–4647 (Aug. 15,

1989); Grem et al., "Phase I Study of Taxol Administered as a Short IV Infusion Daily For 5 Days," Cancer Treatment Reports, Vol. 71 No. 12, (December, 1987); Donehower et al., "Phase I Trial of Taxol in Patients With Advanced Cancer," Cancer Treatment Reports, Vol, 71, No. 12, (December, 1987); Holmes et al., "Phase II Study of Taxol in Patients (PT) with Metastatic Breast Cancer (MBC)," Proceedings of the American Society of Clinical Oncology, Vol. 10, (March, 1991), pp. 60. See also Suffness. "Development of Antitumor Natural Products at the National Cancer Institute," Gann Monograph or Cancer Research, 31 (1989) pp. 21–44 (which recommends that taxol only be given as a 24-hour infusion).

Weiss et al, in "Hypersensitivity Reactions from Taxol," Journal of Clinical Oncology, Vol. 8, No. 7 (July 1990) pp. 1263–1268, reported that it was difficult to determine a reliable overall incidence of hypersensitivity reactions, HSRs, because of the wide variations in taxol doses and schedules used, and the unknown degree of influence that changing the infusion schedule and using premedication has on HSR incidents. For example, of five patients who received taxol in a 3-hour infusion at greater that 190 mg/m^2 with no premedication, three had reactions, while only one out of 30 patients administered even higher doses over a 6-hour infusion with no premedication had a reaction. Therefore, this suggests that prolonging the infusion to beyond 6 hours is sufficient to reduce HSR incidents. Nevertheless, Weiss et al. found that patients receiving 250 mg/m^2 of taxol administered via a 24-hour infusion still had definite HSRs. Thus, while prolonging drug infusion to 6 or 24-hours may reduce the risk for an acute reaction, this conclusion can not be confirmed, since 78% of the HSR reactions occurred within ten minutes of initiating the taxol infusion, which indicates that the length of time planned for the total infusion would have no bearing. Further, concentration of taxol in the infusion may also not make a difference since substantial number of patients had reactions to various small taxol dosages. Finally, not only is the mechanism of taxol HSR unknown, it is also not clear whether taxol itself is inducing HSRs, or if the HSRs are due to the excipient (Cremaphor EL; Badische Anilin und Soda Fabrik AG [BASF], Ludwigshafen, Federal Republic of Germany). Despite the uncertainty as to whether or not premedication had any influence on reducing the severity or number of HSRs, prophylactic therapy was recommended, since there is no known danger from its use.

The conflicting recommendations in the prior art concerning whether premedication should be used to avoid hypersensitivity reactions when using prolonged infusion durations, and the lack of efficacy data for infusions done over a six-hour period has led to the use of a 24-hour infusion of high doses (above 170 mg/m^2) of taxol in a Cremaphor EL emulsion as an accepted cancer treatment protocol.

Although it appears possible to minimize the side effects of administering taxol in an emulsion by one of a long infusion duration, the long infusion duration is inconvenient for patients, and is expensive due to the need to monitor the patients for the entire 6 to 24-hour infusion duration. Further, the long infusion duration requires that patients spend at least one night in a hospital or treatment clinic.

The use of higher doses of paclitaxel has also been described in the literature. To determine the maximal-tolerated dose (MTD) of paclitaxel in combination with high-dose cyclophosphamide and cisplatin followed by autologous hematopoietic progenitor-cell support (AHPCS), Stemmer et al (Stemmer S M, Cagnoni P J, Shpall E J, et al: High-dose paclitaxel, cyclophophamide, and cisplatin with autologous hematopoietic progenitor-cell support: A phase I trial. J Clin Oncol 14:1463–1472, 1996) have conducted a phase I trial in forty-nine patients with poor-prognosis breast cancer, non-Hodgkin's lymphoma (NHL) or ovarian cancer with escalating doses of paclitaxel infused over 24 hours, followed by cyclophosphamide (5,625 mg/m^2) and cisplatin (165 mg/m^2) and AHPCS. Dose-limiting toxicity was encountered in two patients at 825 mg/m^2 of paclitaxel; one patient died of multi-organ failure and the other developed grade 3 respiratory, CNS, and renal toxicity, which resolved. Grade 3 polyneuropathy and grade 4 CNS toxicity were also observed. The MTD of this combination was determined to be paclitaxel (775 mg/m^2), cyclophosphamide (5,625 mg/m^2), and cisplatin (165 mg/m^2) followed by AHPCS. Sensory polyneuropathy and mucositis were prominent toxicities, but both were reversible and tolerable. Eighteen of 33 patients (54%) with breast cancer achieved a partial response. Responses were also observed in patients with NHL (four of five patients) and ovarian cancer (two of two patients).

U.S. Pat. No. 5,641,803 reports the use of Taxol at doses of 175 and 135 mg/m^2, administered in a 3 hour infusion. The infusion protocols require the use of premedication and reports the incidences of hypersensitivity reactions in 35% of the patients. Neurotoxicity was reported in 51% of the patients, with 66% of patients experiencing neurotoxicity in the high dose group and 37% in the low dose group. Furthermore, it was noted that 48% of the patients experienced neurotoxicity for longer infusion times of 24 hours while 54% of patients experienced neurotoxicity for the shorter 3 hour infusion.

There is evidence in the literature that higher doses of paclitaxel result in a higher response rate. The optimal doses and schedules for paclitaxel are still under investigation. To assess the possibility that paclitaxel dose intensity may be important in the induction of disease response, Reed et al of NCI (Reed E, Bitton R, Sarosy G, Kohn E: Paclitaxel dose intensity. Journal of Infusional Chemotherapy 6:59–63, 1996) analyzed the available phase II trial data in the treatment of ovarian cancer and breast cancer. Their results suggest that the relationship between objective disease response and paclitaxel dose intensity in recurrent ovarian cancer is highly statistically significant with two-side p value of 0.022. The relationship in breast cancer is even stronger, with a two-sided p value of 0.004. At 135 mg/m^2/21 days, the objective response rate was 13.2%; and at 250 mg/m^2/21 days, the objective response rate was 35.9%. The response rate seen at the intermediate dose of 175 mg/m^2 was linear with the 135 mg/m^2 and 250 mg/m^2 results and the linear regression analysis shows a correlation coefficient for these data of 0.946 (Reed et al, 1996).

In a study by Holmes (Holmes F A, Wasters R S, Theriault R L, et al: Phase II trial of Taxol, an active drug in the treatment of metastatic breast cancer. J Natl Cancer Inst 83:1797–1805, 1991), and at MSKCC (Reichman B S, Seidman A D, Crown J P A, et al: Paclitaxel and recombinant human granulocyte colony-stimulating factor as initial chemotherapy for metastatic breast cancer. J Cin Oncol 11:1943–1951, 1993), it was shown that higher doses of TAXOL up to 250 mg/m^2 produced greater responses (60%) than the 175 mg/m^2 dose (26%) currently approved for TAXOL. These results, however, have not been reproduced due to higher toxicity at these higher doses. These studies, however, bear proof to the potential increase in response rate at increased doses of paclitaxel.

Since premedication is required for the administration of Taxol, often necessitating overnight stays of the patient at

the hospital, it is highly desirable to develop formulations of paclitaxel that obviate the need for premedication.

Since premedication is required for the administration of Taxol, due to HSR's associated with administration of the drug, it is highly desirable to develop a formulation of paclitaxel that does not cause hypersensitivity reactions. It is also desirable to develop formulations of paclitaxel that do not cause neurotoxicity.

Since Taxol infusions are generally preceded by premedication, and require post-infusion monitoring and record keeping, often necessitating overnight stays of the patient at the hospital, it is highly desirable to develop a formulation of paclitaxel which would allow for recipients to be treated on an out-patient basis.

Since it has been demonstrated that higher doses of Taxol achieve improved clinical responses albeit with higher toxicity, it is desirable to develop a formulation of paclitaxel which can achieve these doses without this toxicity.

Since it has been demonstrated that the dose limiting toxicity of Taxol is cerebral and neurotoxicity, it is desirable to develop a formulation of paclitaxel that decreases such toxicity.

It is also desirable to eliminate the need to use premedication since this increases patient discomfort and increases the expense and duration of treatment.

It is also desirable to shorten the duration required for the infusion of Taxol (currently administered in 3–24 hours) to minimize patient stay at the hospital or clinc.

Since Taxol is currently approved for administration at concentrations between 0.6–1.2 mg/ml and a typical dose in humans is about 250–350 mg, this results in infusion volumes typically greater than 300 ml. It is desirable to reduce these infusion volumes. This can be done by the development of formulations of paclitaxel that are stable at higher concentrations so as to reduce the time of administration.

BREIF DESCRIPTION OF THE INVENTION

The anticancer agent paclitaxel (TAXOL, Bristol Myers Squibb, BMS,) has remarkable clinical activity in a number of human cancers including cancers of the ovary, breast, lung, esophagus, head and neck region, bladder and lymphomas. It is currently approved for the treatment of ovarian carcinoma where it is used in combination with cisplatin and for metastatic breast cancer that has failed prior treatment with one combination chemotherapy regimen. The major limitation of Taxol is its poor solubility and consequently the BMS formulation contains 50% Cremaphor EL and 50% ethanol as the solubilizing vehicle. Prior to intravenous administration, this formulation must be diluted 1:10 in saline for a final dosing solution containing 0.6 mg/ml of paclitaxel. This formulation has been linked to severe hypersensitivity reactions in animals (Lorenz et al., *Agents Actions* 1987, 7, 63–67) and humans (Weiss et al., *J. Clin. Oncol.* 1990, 8, 1263–63) and consequently requires premedication of patients with corticosteroids (dexamethasone) and antihistamines. The large dilution results in large volumes of infusion (typical dose 175 mg/m^2) upto 1 liter and infusion times ranging from 3 hours to 24 hours. Thus, there is a need for an alternative less toxic formulation for paclitaxel.

Capxol™ is a novel, cremophor-free formulation of the anticancer drug paclitaxel. The inventors, based on animal studies, believe that a cremophor-free formulation will be significantly less toxic and will not require premedication of patients. Premedication is necessary to reduce the hypersensitivity and anaphylaxis that occurs as a result of cremophor in the currently approved and marketed BMS (Bristol Myers Squibb) formulation of paclitaxel. Capxol™ is a lyophilized powder for reconstitution and intravenous administration. When reconstituted with a suitable aqueous medium such as 0.9% sodium chloride injection or 5% dextrose injection, Capxol™ forms a stable colloidal solution of paclitaxel. The size of the collodial suspension may range from 20 nm to 8 microns with a preferred range of about 20–400 nm. The two major components of Capxol™ are unmodified paclitaxel and human serum albumin (HSA). Since HSA is freely soluble in water, Capxol™ can be reconstituted to any desired concentration of paclitaxel limited only by the solubility limits for HSA. Thus Capxol™ can be reconstituted in a wide range of concentrations ranging from dilute (0.1 mg/ml paclitaxel) to concentrated (20 mg/ml paclitaxel). This can result in fairly small volumes of administration.

In accordance with the present invention, there are provided compositions and methods useful for in vivo delivery of biologics, in the form of nanoparticles that are suitable for parenteral administration in aqueous suspension. Invention compositions comprise drugs, such as paclitaxel, stabilized by a polymer. The polymer is a biocompatible material, such as the protein albumin. Use of invention compositions for the delivery of biologics obviates the necessity for administration of biologics in toxic diluents of vehicles, for example, ethanol and polyethoxylated 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 compositions is their propensity to produce severe allergic and other side effects.

It is known that the delivery of biologics in the form of a particulate suspension allows targeting to organs such as the liver, lungs, spleen, lymphatic circulation, and the like, due to the uptake in these organs, of the particles by the reticuloenodothelial (RES) system of cell. Targeting to the RES containing organs may be controlled through the use of particles of varying size, and through administration by different routes. But when administered to rats, Capxol was unexpectedly and surprisingly found to accumulate in tissues other than those containing the RES such as the prostate, pancreas, testes, seminiferous tubules, bone, etc. to a significantly greater level than Taxol at similar doses.

Thus, it is very surprising that the invention formulation of paclitaxel, Capxol, a nanoparticle formulation, concentrates in tissues such as the prostate, pancreas, testes, seminiferous tubules, bone, etc., i.e., in organs not containing the RES, at a significantly higher level than a non-particulate formulation of paclitaxel such as Taxol. Thus, Capxol may be utilized to treat cancers of these tissues with a higher efficacy than Taxol. However, the distribution to many other tissues is similar for Capxol and Taxol, therefore Capxol is expected to maintain anticancer activity at least equal to that of TAXOL in other tissues.

The basis for the localization within the prostate could be a result of the particle size of the formulation (20–400 nm), or the presence the protein albumin in the formulation which may cause localization into the prostatic tissue through specific membrane receptors (gp 60, gp 18, gp 13 and the like). It is also likely that other biocompatible, biodegradable polymers other than albumin may show specificity to certain tissues such as the prostate resulting in high local concentration of paclitaxel in these tissues as a result of the properties described above. Such biocompatible materials are contemplated within the scope of this invention. A

preferred embodiment of a composition to achieve high local concentrations of paclitaxel in the prostate is a formulation containing paclitaxel and albumin with a particle size in the range of 20–400 nm, and free of cremophor. This embodiment has also been demonstrated to result in higher level concentrations of paclitaxel in the, pancreas, kidney, lung, heart, bone, and spleen when compared to Taxol at equivalent doses. These properties provide novel applications of this formulation of paclitaxel including methods of lowering testosterone levels, achieving medical orchiectomy, providing high local concentrations to coronary vasculature for the treatment of restenosis.

It is also very surprising that paclitaxel is metabolized into its metabolites at a much slower rate than Taxol when administered as Capxol. This enables increased and sustained anticancer activity for longer periods with similar doses of paclitaxel.

It is also very surprising that when Capxol and Taxol are administered to rats at equivalent doses of paclitaxel, a much higher degree of myelosuppression results for the Taxol group compared to the Capxol group. This can result in lower indicidences of infections and fever episodes (e.g., febrile neutropenia). It can also reduce the cycle time in between treatments which is currently 21 days. Thus the use of Capxol may provide substantial advantage over Taxol.

It was surprisingly found that the Taxol vehicle, Cremophor/Ethanol diluted in saline, alone caused severe hypersensitivity reactions and death in several dose groups of mice. No such reactions were observed for the Capxol groups at equivalent and higher doses. Thus Capxol, a formulation of paclitaxel that is free of the Taxol vehicle is of substantial advantage.

It is also very surprising that when Capxol and Taxol are administered to rats at equivalent doses of paclitaxel, a much lower toxicity is seen for the Capxol compared to Taxol as evidenced by significantly higher LD50 values. This may allow for higher more therapeutically effective doses of paclitaxel to be administered to patients. There is evidence in the literature showing increases response rates to higher doses of paclitaxel. The Capxol formulation may allow the administration of these higher doses due to lower toxicity and thereby exploit the full potential of this drug.

Surprisingly, the Capxol formulations show an increased efficacy when compared to TAXOL. In addition, higher doses of paclitaxel are achieved in the Capxol groups due to lower toxicity of the formulation. These high doses can be administered as bolus injections.

It is also surprising that Capxol, a formulation of the substantially water-insoluble drug, paclitaxel, is stable when reconstituted in an aqueous medium at several different concentrations ranging from, but not limited to 0.1–20 mg/ml. This offers substantial advantage over Taxol during administration of the drug as it results in smaller infusion volumes, overcomes instability issues known for Taxol, such as precipitation, and avoids the use of an in-line filter in the infusion line. Thus Capxol greatly simplifies and improves the administration of paclitaxel to patients.

It is also surprising that Capxol when administered to rats at equivalent doses of paclitaxel as Taxol, shows no sign of neurotoxicity while Taxol even at low doses shows neurotoxic effects.

The invention formulation further allows the administration of paclitaxel, and other substantially water insoluble pharmacologically active agents, employing a much smaller volume of liquid and requiring greatly reduced administration time relative to administration volumes and times required by prior art delivery systems.

In combination with a biocompatible polymer matrix, the invention formulation (Capxol) allows for local sustained delivery of paclitaxel with lower toxicity and prolonged activity.

The above surprising findings for Capxol offer the potential to substantially improve the quality of life of patients receiving paclitaxel.

Potential Advantages of the Capxol™ formulation for Paclitaxel

Capxol™ is a lyophilized powder containing paclitaxel and human serum albumin. Due to the nature of the colloidal solution formed from reconstitution of the lyophilized powder toxic emulsifiers such as cremophor (in the BMS formulation of paclitaxel) or polysorbate 80 (as in the Rhone Poulenc formulation of docetaxel) and solvents such as ethanol to solubilize the drug are not required. Removing toxic emulsifiers will reduce the incidences of severe hypersensitivity and anaphylactic reactions that are known to occur in products TAXOL.

- In addition, no premedication with steroids and antihistamines are anticipated prior to administration of the drug.
- Due to reduced toxicities, as evidenced by the LD_{10}/__ LD_{50} studies, higher doses may be employed for greater efficacy.
- The reduction in myelosuppression (as compared with the BMS formulation) is expected to reduce the period of the treatment cycle (currently 3 weeks) and improve the therapeutic outcomes.
- Capxol™ can be administered at much higher concentrations (up to 20 mg/ml) compared with the BMS formulation (0.6 mg/ml), allowing much lower volume infusions, and administration as an intravenous bolus.
- TAXOL may be infused only with nitroglycerin polyolefin infusion sets due to leaching of plasticizers from standard infusion tubing into the formulation. Capxol shows no leaching and may be utilized with any standard infusion tubing. In addition, only glass or polyolefin containers are to be used for storing all cremophor containing solutions. The Capxol formulation has no such limitations.
- A recognized problem with TAXOL formulation is the precipitation of paclitaxel in indwelling catheters. This results in erratic and poorly controlled dosing. Due to the inherent stability of the colloidal solution of the new formulation, Capxol™, the problem of precipitation is alleviated.
- The administration of Taxol requires the use of in line filters to remove precipitates and other particulate matter. Capxol has no such requirement due to inherent stability.
- The literature suggests that particles in the low hundred nonometer size range preferentially partition into tumors through leaky blood vessels at the tumor site. The colloidal particles of paclitaxel in the Capxol™ formulation may therefore show a preferential targeting effect, greatly reducing the side effects of paclitaxel administered in the BMS formulation.

Therefore, it is a primary object of the present invention to provide a new formulation of paclitaxel that provides the above desirable characteristics.

It is another object of the present invention to provide a new formulation of paclitaxel that localizes paclitaxel in certain tissues, thereby providing higher anticancer activity at these sites.

It is another object of the invention to administer paclitaxel at concentrations greater than about 2 mg/ml in order to reduce infusion volumes.

It is also an object of the invention to provide a formulation of paclitaxel that is free of the Taxol vehicle.

It is yet another object of the invention to provide a formulation of paclitaxel that improves the quality of life of patients receiving Taxol for the treatment of cancer.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided compositions for in vivo delivery of a biologic. As used herein, the term "in vivo delivery" refers to delivery of a biologic by such routes of administration as oral, intravenous, subcutaneous, intrapertioneal, intrathecal, intramuscular, intracranial, inhalational, topical, transdermal, suppository (rectal), pessary (vaginal), and the like.

As used herein, the term "biologic" refers to pharmaceutically active agents (such as analgesic agents, anesthetic agents, anti-asthamatic agents, antibiotics, anti-depressant agents, anti-diabetic agents, anti-fungal agents, anti-hypertensive agents, anti-inflammatory agents, anti-neoplastic agents, anxiolytic agents, enzymatically active agents, nucleic acid constructs, immunostimulating agents, immunosuppressive agents, physiologically active gases, vaccines, and the like), diagnostic agents (such as ultrasound contrast agents, radiocontrast agents, or magnetic contrast agents), agents of nutritional value, and the like.

As used herein, the term "micron" refers to a unit of measure of one one-thousandth of a millimeter. The term "nono-" refers to dimensions that are less than 1 micron.

A number of biocompatible material may be employed in the practice of the present invention for the formation of a plymeric shell. As used herein, the term "biocompatible" describes a substance that does not appreciably alter or affect in any adverse way, the biological system into which it is introduced. A presently preferred polymeric for use in the formation of a shell is the protein albumin. Other suitable biocompatible material maybe utilized in the present formulation and these have been discussed in detail in related applications.

Several biocompatible materials may be employed in the practice of the present invention for the formation of a polymeric shell. For example, naturally occurring biocompatible materials such as proteins, polypeptides, oligopeptides, polynucleotides, polysaccharides (e.g., starch, cellulose, dextrans, alginates, chitosan, pectin, hyaluronic acid, and the like), lipids, and so on, are candidates for such modification.

As examples of suitable biocompatible materials, naturally occurring or synthetic proteins may be employed, Examples of suitable proteins include albumin (which contains 35 cysteine residues), insulin (which contains 6 cysteines), hemoglobin (which contains 6 cysteine residues per $a_2\beta_2$ unit), lysozyme (which contains 8 cysteine residues), immunoglobulins, a-2-macroglobulin, fibronectin, vitronectin, fibrinogen, casein and the like, as well as combinations of any two or more thereof.

A presently preferred protein for use in the formation of a polymeric shell is albumin. Optionally, proteins such as a-2-macroglobulin, a known opsonin, could be used to enhance uptake of the shell encased particles of biologic by macrophase-like cells, or to enhance the uptake of the shell encased particles into the liver and spleen. Other ligands such as glycoproteins may also enhance uptake into certain tissues. Other functional proteins, such as antibodies or enzymes, which could facilitate targeting of biologic to a desired site, can also be used in the formation of the polymeric shell.

Similarly, synthetic polymers are also good candidates for preparation of the drug formulation. Examples include polyalkylene glycols (e.g., linear or branched chain), polyvinyl alcohol, polyacrylates, polyhydroxyethyl methacrylate, polyacrylic acid, polyethyloxazoline, polyacrylamides, polyisopropyl acrylamides, polyvinyl pyrrolidinone, polylactide/glycolide and the like, and combinations thereof, are good candidates for the biocompatible polymer in the invention formulation.

These biocompatible materials may also be employed in several physical forms such as gels, crosslinked or uncrosslinked to provide matrices from which the pharmacologically active ingredient, for example paclitaxel, may be released by diffusion and/or degradation of the matrix. Temperature sensitive materials may also be utilized as the dispersing matrix for the invention formulation. Thus for example, the Capxol may be injected in a liquid formulation of the temperature sensitive material (e.g., copolymers of polyacrylamides or copolymers of polyalkylene glycols and polylactide/glycolides) which gel at the tumor site and provide slow release of Capxol. The Capxol formulation may be dispersed into a matrix of the above mentioned biocompatible polymers to provide a controlled release formulation of paclitaxel, which through the properties of the Capxol formulation (albumin associated with paclitaxel) results in lower toxicity to brain tissue as well as lower systemic toxicity as discussed below. This combination of Capxol or other chemotherapeutic agents formulated similar to Capxol together with a biocompatible polymer matrix may be useful for the controlled local delivery of chemotherapeutic agents for treating solid tumors in the brain and peritoneum (ovarian cancer) and in local applications to other solid tumors. These combination formulations are not limited to the use of paclitaxel and may be utilized with a wide variety of pharmacologically active ingredients including antiinfectives, immunosuppressives and other chemotherapeutics and the like.

In the preparation of invention compositions, one can optionally employ a dispersing agent to suspend or dissolve biologic. Dispersing agents contemplated for use in the practice of the present invention include any liquid that is capable of suspending or dissolving biologic, but does not chemically react with either the polymer employed to produce the shell, or the biologic itself. Examples include water, vegetable oils, (e.g., soybean oil, mineral oil, corn oil, rapeseed oil, coconut oil, olive oil, safflower oil, cotton seed oil, and the like), aliphatic, cycloaliphatic, or aromatic hydrocarbons having 4–30 carbon atoms (e.g., n-dodecane, n-decane, n-hexane, cyclohexane, toluene, benzene, and the like), aliphatic or aromatic alcohols having 1–30 carbon atoms (e.g., octanol, and the like), aliphatic or aromatic esters having 2–30 carbon atoms (e.g., ethyl capyrlate (octanoate), and the like), alkyl, aryl, or cyclic ethers having 2–30 carbon atoms (e.g., diethyl ether, tetrahydrofuran, and the like), alkyl or aryl halides having 1–30 carbon atoms (and optionally more than one halogen substituent, e.g., CH_3Cl, CH_2Cl_2, $CHCl_3$, $CH_2Cl—CH_2Cl$, and the like), ketones having 3–30 carbon atoms (e.g., acetone, methyl ethyl ketone, and the like), polyalkylene glycols (e.g., polyethylene glycol, and the like), or combinations of any two or more thereof.

Especially preferred combinations of dispersing agents include volatile liquids such as dichloromethane, chloroform, ethyl acetate, benzene, and the like (i.e., solvents that have a high degree of solubility for the pharma-

cologically active agent, and are soluble in the other dispersing agent employed), along with a less volatile dispersing agent. When added to the other dispersing agent, these volatile additives help to drive the solubility of the pharmacologically active agent into the dispersing agent. This is desirable since this step is usually time consuming. Following dissolution, the volatile component may be removed by evaporation (optionally under vacuum).

Particles of biologic substantially completely contained within a polymeric shell, or associated therewith, prepared as described herein, are delivered neat, or optionally as a suspension in a biocompatible medium. This medium may be selected from water, buffered aqueous media, saline, buffered saline, optionally buffered solutions of amino acids, optionally buffered solutions of proteins, optionally buffered solutions of sugars, optionally buffered solutions of carbohydrates, optionally buffered solutions of vitamins, optionally buffered solutions of synthetic polymers, lipid-containing emulsions, and the like.

In addition, the polymeric shell can optionally be modified by a suitable agent, wherein the agent is associated with the polymeric shell through an optional covalent bond. Covalent bonds contemplated for such linkages include ester, ether, urethane, diester, amide, secondary or tertiary amine, phosphate ester, sulfate ester, and the like bonds. Suitable agents contemplated for this optional modification of the polymeric shell include synthetic polymers (polyalkylene glycols (e.g., linear or branched chain polyethylene glycol), polyvinyl alcohol, polyhydroxyethyl methacrylate, polyacrylic acid, polyethyloxazoline, polyacrylamide, polyvinyl pyrrolidinone, and the like), phospholipids (such as phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI), sphingomyelin, and the like), proteins (such as enzymes, antibodies, and the like), polysaccharides (such as starch, cellulose, dextrans, alginates, chitosan, pectin, hyaluronic acid, and the like), chemical modifying agents (such as pyridoxal 5'-phosphate, derivatives of pyridoxal, dialdehydes, diaspirin esters, and the like), or combinations of any two or more thereof.

Variations on the general theme of dissolved biologic enclosed within a polymeric shell are possible. A suspension of fine particles of biologic in a biocompatible dispersing agent could be used (in place of a biocompatible dispersing agent containing dissolved biologic) to produce a polymeric shell containing dispersing agent-suspended particles of biologic. In other words, the polymeric shell could contain a saturated solution of biologic in dispersing agent. Another variation is a polymeric shell containing a solid core of biologic produced by initially dissolving the biologic in a volatile organic solvent (e.g. benzene), forming the polymeric shell and evaporating the volatile solvent under vacuum, e.g., in an evaporator, spray drier or freeze-drying the entire suspension. This results in a structure having a solid core of biologic surrounded by a polymer coat. This latter method is particularly advantageous for delivering high doses of biologic in a relatively small volume. In some cases, the biocompatible material forming the shell about the core could itself be a therapeutic or diagnostic agent, e.g., in the case of insulin, which may be delivered as part of a polymeric shell formed in the process described above. In other cases, the polymer forming the shell could participate in the delivery of a biologic, e.g., in the case of antibodies used for targeting, or in the case of hemoglobin, which may be delivered as part of a polymeric shell formed in the ultrasonic irradiation process described above, thereby providing a blood substitute having a high binding capacity for oxygen.

In accordance with a specific embodiment of the present invention, there are provided pharmaceutically acceptable formulations of paclitaxel useful for the treatment of primary tumors in a subject, which formulations achieve high local concentrations of paclitaxel at the tumor site, wherein the invention formulations are substantially free of cremophor. Primary tumors contemplated for treatment with invention formulations include cancers of prostate, testes, lung, kidney, pancreas, bone, spleen, liver, brain, and the like.

In accordance with another embodiment of the present invention, there are provided pharmaceutically acceptable formulations of paclitaxel useful for the treatment of brain tumors in a subject, which formulations achieve high local concentrations of paclitaxel at the tumor site, and wherein said formulations are substantially free of cremophor, thereby inducing reduced cerebral and/or neurologic toxicity.

Invention formulations are useful for the treatment of a variety of indications, e.g., brain tumors, intraperitoneal tumors, prostatitis, bph, restenosis, atherosclerosis, and the like. Invention compositions have been observed to reduce the rate of metabolism of paclitaxel (relative to the rate of metabolism when paclitaxel is formulated for delivery as described in the prior art, e.g., as Taxol), thus a higher activity remains 24 hrs after administration.

In accordance with yet another embodiment of the present invention, there are provided pharmaceutically acceptable formulations of paclitaxel useful for the reduction of serum testosterone levels (low dose paclitaxel) in a subject. Such formulations are useful for the treatment of various urogenital disorders.

Paclitaxel-containing formulations according to the invention can be lyophilized, and conveniently reconstituted at concentrations greater than about 1.2 mg/ml (with concentrations greater than about 2 mg/ml preferred, and concentrations greater than about 3 mg/ml being especially preferred). The resulting reconstituted materials are stable for at least 3 days. Another advantage of paclitaxel-containing formulations according to the invention is their suitability for administration using standard i.v. infusion tubing (i.e., there is no need to use specialized tubing to delivery paclitaxel).

Paclitaxel-containing formulations according to the invention can be administered employing relatively small volumes for delivery, e.g., typically requiring infusion volumes <200 ml for a therapeutic dose. In addition, infusion can typically be accomplished over a relatively short period of time, e.g., over about 2–3 hrs, delivering doses >about 250–300 mg/m^2.

Because invention formulations can be delivered in substantially higher concentrations than heretofor available in the art, and over substantially reduced time periods, use of invention formulations frequently eliminates the necessity for a patient to remain under direct medical observation for extended periods of time.

In accordance with yet another embodiment of the present invention, there are provided methods for the administration of paclitaxel to a subject in need thereof, said methods comprising systemically administering a therapeutically effective amount of paclitaxel to said subject in a pharmaceutically acceptable formulation without the use of premedication, wherein said paclitaxel can optionally be administered as a bolus injection.

As readily recognized by those of skill in the art, invention compositions can be administered over a variety of

time-frames. Of course it is recognized that the more quickly a medicament can be delivered to a patient, the less intrusive the procedure will be. Accordingly, it is presently preferred that the administration period is no greater than about 1 hour, and that the treatment cycle last no greater than about 2 weeks.

Suitable therapeutically effective doses can readily be determined by those of skill in the art, typically falling in the range of about 135 mg/m^2, with doses of at least about 175 mg/m^2 being presently preferred and doses of at least about 200 mg/m^2 being especially preferred.

In accordance with a particularly preferred aspect of the present invention, there are provided methods for reducing the hematologic toxicity of paclitaxel in a subject undergoing treatment therewith, said methods comprising systemically administering paclitaxel to said subject in a pharmaceutically acceptable formulation, as described herein. Preferably, such formulations are substantially free of cremophor.

In accordance with another particularly preferred aspect of the present invention, there are provided methods for reducing the cerebral or neurologic toxicity of paclitaxel in a subject undergoing treatment therewith, said methods comprising systemically administering said paclitaxel to said subject in a pharmaceutically acceptable formulation as described herein. Preferably, such formulations are substantially free of cremophor.

In accordance with yet another particularly preferred aspect of the present invention, there are provided methods for the treatment of primary tumors in a subject by achieving high local concentration of paclitaxel at the tumor site, said methods comprising systemically administering paclitaxel to said subject in a pharmaceutically acceptable formulation free of cremophor. Primary tumors contemplated for treatment by invention methods include cancers of prostate, testes, lung, kidney, pancreas, bone, spleen, liver, brain, and the like.

In accordance with still another embodiment of the present invention, there are provided unit dosage forms comprising a vessel containing a sufficient quantity of paclitaxel to allow systemic administration at a dose of at least 135 mg/m^2 over an administration period of no greater than 2 hours. As readily recognized by those of skill in the art, paclitaxel used for the preparation of such unit dosage forms can be in aqueous media, a non-aqueous formulation of paclitaxel, a dry powder formulation of paclitaxel, and the like.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1

Preparation of Protein Shell Containing Oil

Three ml of a USP (United States Pharmacopoeia) 5% human serum albumin solution (Alpha Therapeutic Corporation) were taken in a cylindrical vessel that could be attached to a sonicating probe (Heat Systems, Model XL2020). The albumin solution was overlayed with 6.5 ml of USP grade soybean oil (soya oil). The tip of the sonicator probe was brought to the interface between the two solutions and the assembly was maintained in a cooling bath at 20° C. The system was allowed to equilibrate and the sonicator turned on for 30 seconds. Vigorous mixing occurred and a white milky suspension was obtained. The suspension was diluted 1:5 with normal saline. A particle counter (Particle Data Systems, Elzone, Model 280 PC) was utilized to determine size distribution and concentration of oil-containing protein shells. The resulting protein shells were determined to have a maximum cross-sectional dimension of about 1.35±0.73 microns, and the total concentration determined to be ~10^9 shells/ml in the original suspension.

As a control, the above components, absent the protein, did not form a stable microemulsion when subjected to ultrasonic irradiation. This result suggests that the protein is essential for formation of microspheres. This is confirmed by scanning electron micrograph and transmission electron micrograph studies as described below.

Example 2

Preparation of Polymeric Shells Containing Dissolved Taxol

Taxol was dissolved in USP grade soybean oil at a concentration of 2 mg/ml. 3 ml of a USP 5% human serum albumin solution was taken in a cylindrical vessel that could be attached to a sonicating probe. The albumin solution was overlayed with 6.5 ml of soybean oil/taxol solution. The tip of the sonicator probe was brought to the interface between the two solutions and the assembly was maintained in equilibrium and the sonicator turned on for 30 seconds. Vigorous mixing occurred and a stable white milky suspension was obtained which contained protein-walled polymeric shells enclosing the oil/taxol solution.

In order to obtain a higher loading of drug into the crosslinked protein shell, a mutual solvent for the oil and the drug (in which the drug has a considerably higher solubility) can be mixed with the oil. Provided this solvent is relatively non-toxic (e.g., ethyl acetate), it may be injected along with the original carrier. In other cases, it may be removed by evaporation of the liquid under vacuum following preparation of the polymeric shells.

It is recognized that several different methods may be employed to achieve the physical characteristics of the Capxol formulation. The biological properties associated with this formulation of higher local concentrations at specific organ sites (prostate, lung, pancreas, bone, kidney, heart) as well as lower toxicities (increased LD50, decreased myelosuppression, decreased cerebral toxicity) associated with higher efficacies is independent of the method of manufacture.

Example 3

In Vivo Biodistribution—Crosslinked Protein Shells Containing a Fluorophore

To determine the uptake and biodistribution of liquid entrapped within protein polymeric shells after intravenous injection, a fluorescent dye (rubrene, available from Aldrich) was entrapped within a human serum albumin (HSA) protein polymeric shell and used as a marker. Thus, rubrene was dissolved in toluene, and albumin shells containing toluene/rubrene were prepared as described above by ultrasonic irradiation. The resulting milky suspension was diluted five times in normal saline. Two ml of the diluted suspension was then injected into the tail vein of a rat over 10 minutes. One animal was sacrificed an hour after injection and another 24 hours after injection.

100 micron frozen sections of lung, liver, kidney, spleen, and bone marrow were examined under a fluorescent microscope for the presence of polymeric shell-entrapped fluorescent dye or released dye. At one hour, the majority of the polymeric shells appeared to be intact (i.e., appearing as

brightly fluorescing particles of about 1 micron diameter), and located in the lungs and liver. At 24 hours, the dye was observed in the liver, lungs, spleen, and bone marrow. A general straining of the tissue was also observed, indicating that the shell wall of the polymeric shells had been digested, and the dye liberated from within. This result was consistent with expectations and demonstrates the potential use of invention compositions for delayed or controlled release of an entrapped pharmaceutical agent such as taxol.

Example 4

Toxicity of Polymeric Shells Containing Soybean Oil (SBO)

Polymeric shells containing soybean oil were prepared as described in Example 1. The resulting suspension was diluted in normal saline to produce two different solutions, one containing 20% SBO and the other containing 30% SBO.

Intralipid, a commercially available TPN agent, contains 20% SBO. The LD_{50} for Intralipid in mice is 120 ml/kg, or about 4 ml for a 30 g mouse, when injected at 1 cc/min.

Two groups of mice (three mice in each group; each mouse weighing about 30 g) were treated with invention composition containing SBO as follows. Each mouse was injected with 4 ml of the prepared suspension of SBO-containing polymeric shells. Each member of one group received the suspension containing 20% SBO, while each member of the other group received the suspension containing 30% SBO.

All three mice in the group receiving the suspension containing 20% SBO survived such treatment, and showed no gross toxicity in any tissues or organs when observed one week after SBO treatment. Only one of the three mice in the group receiving suspension containing 30% SBO died after injection. These results clearly demonstrate that oil contained within polymeric shells according to the present invention in not toxic at its LD_{50} dose, as compared to a commercially available SBO formulation (Intralipid). This effect can be attributed to the slow release (i.e., controlled rate of becoming bioavailable) of the oil from within the polymeric shell. Such slow release prevents the attainment of a lethal dose of oil, in contrast to the high oil dosages attained with commercially available emulsions.

Example 5

In vivo Bioavailability of Soybean Oil Released from Polymeric Shells

A test was performed to determine the slow or sustained release of polymeric shell-enclosed material following the injection of a suspension of polymeric shells into the blood stream of rats. Crosslinked protein (albumin) walled polymeric shells containing soybean oil (SBO) were prepared by sonication as described above. The resulting suspension of oil-containing polymeric shells was diluted in saline to a final suspension containing 20% oil. Five ml of this suspension was injected into the cannulated external jugular vein of rats over a 10 minute period. Blood was collected from these rats at several time points following the injection and the level of triglycerides (soybean oil is predominantly triglyceride) in the blood determined by routine analysis.

Five ml of a commercially available fat emulsion (Intralipid, an aqueous parenteral nutrition agent—containing 20% soybean oil, 1.2% egg yolk phospholipids, and 2.25% glycerin) was used as a control. The control utilizes egg phosphatide as an emulsifier to stabilize the emulsion. A comparison of serum levels of the triglycerides in the two cases would give a direct comparison of the bioavailability of the oil as a function of time. In addition to the suspension of polymeric shells containing 20% oil, five ml of a sample of oil-containing polymeric shells in saline at a final concentration of 30% oil was also injected. Two rats were used in each of the three groups. The blood levels of triglycerides in each case are tabulated in Table 1, given in units of mg/dl.

TABLE 1

	SERUM TRIGLYCERIDES (mg/dl)					
GROUP	Pre	1 hr	4 hr	24 hr	48 hr	72 hr
Intralipid Control (20% SBO)	11.4	941.9	382.9	15.0	8.8	23.8
Polymeric Shells (20% SBO)	24.8	46.7	43.8	29.3	24.2	43.4
Polymeric Shells (30% SBO)	33.4	56.1	134.5	83.2	34.3	33.9

Blood levels before injection are shown in the column marked 'Pre'. Clearly, for the Intralipid control, very high triglyceride levels are seen following injection. Triglyceride levels are then seen to take about 24 hours to come down to preinjection levels. Thus the oil is seen to be immediately available for metabolism following injection.

The suspension of oil-containing polymeric shells containing the same amount of total oil as Intralipid (20%) show a dramatically different availability of detectible triglyceride in the serum. The level rises to about twice its normal value and is maintained at this level for many hours, indicating a slow or sustained release of triglyceride into the blood at levels fairly close to normal. The group receiving oil-containing polymeric shells having 30% oil shows a higher level of triglycerides (concomitant with the higher administered dose) that falls to normal within 48 hours. Once again, the blood levels of triglyceride do not rise astronomically in this group, compared to the control group receiving Intralipid. This again, indicates the slow and sustained availability of the oil from invention composition, which has the advantages of avoiding dangerously high blood levels of material contained within the polymeric shells and availability over an extended period at acceptable levels. Clearly, drugs delivered within polymeric shells of the present invention would achieve these same advantages.

Such a system of soybean oil-containing polymeric shells could be suspended in an aqueous solution of amino acids, essential electrolytes, vitamins, and sugars to form a total parenteral nutrition (TPN) agent. Such a TPN cannot be formulated from currently available fat emulsions (e.g., Intralipid) due to the instability of the emulsion in the presence of electrolytes.

Example 6

Preparation of Protein-walled Polymeric Shells Containing a Solid Core of Pharmaceutically Active Agent

Another method of delivering a poorly water-soluble drug such as taxol within a polymeric shell is to prepare a shell of polymeric material around a solid drug core. Such a 'protein coated' drug particle may be obtained as follows. The procedure described in Example 2 is repeated using an organic solvent to dissolve taxol at a relatively high con-

centration. Solvents generally used are organics such as benzene, toluene, hexane, ethyl ether, chloroform, alcohol and the like. Polymeric shells are produced as described in Example 1. Five ml of the milky suspension of polymeric shells containing dissolved taxol are diluted to 10 ml in normal saline. This suspension is placed in a rotary and the volatile organic removed by vacuum. The resultant suspension is examined under a microscope to reveal opaque cores, indicating removal of substantially all organic solvent, and the presence of solid taxol. The suspension can be frozen and stored indefinitely and used directly or lyophilized at a later time.

Alternatively, the polymeric shells with cores of organic solvent-containing dissolved drug are freeze-dried to obtain a dry crumbly powder that can be resuspended in saline (or other suitable liquid) at the time of use. Although the presently preferred protein for use in the formation of the polymeric shell is albumin, other proteins such as a-2-macroglobulin, a known opsonin, could be used to enhance uptake of the polymeric shells by macrophage-like cells. Alternatively, molecules like PEG could be incorporated into the particles to produce a polymeric shell with increased circulation time in vivo.

Example 7

Targeting of Immunosuppressive Agent to Transplanted Organs Using Intravenous Delivery of Polymeric Shells Containing Such Agents

Immunosuppressive agents are extensively used following organ transplantation for the prevention of rejection episodes. In particular, cyclosporine, a potent immunosuppressive agent, prolongs the survival of allogeneic transplants involving skin, heart, kidney, pancreas, bone marrow, small intestine, and lung in animals. Cyclosporine has been demonstrated to suppress some humoral immunity and to a greater extent, cell mediated reactions such as allograft rejection, delayed hypersensitivity, experimental allergic encephalomyelitis, Freund's adjuvant arthritis, and graft versus host disease in many animal species for a variety of organs. Successful kidney, liver and heart allogeneic transplants have been performed in humans using cyclosporine.

Cyclosporine is currently delivered in oral form either as capsules containing a solution of cyclosporine in alcohol, and oils such as corn oil, polyoxyethylated glycerides and the like, or as a solution in olive oil, polyoxyethylated glycerides, and the like. It is also administered by intravenous injection, in which case it is dissolved in a solution of ethanol (approximately 30%) and Cremaphor (polyoxyethylated castor oil) which must be diluted 1:20 to 1:100 in normal saline or 5% dextrose prior to injection. Compared to an intravenous (i.v.) infusion, the absolute bioavailability of the oral solution is approximately 30% (Sandoz Pharmaceutical Corporation, Publication SDI-Z10 (A4), 1990). In general, the i.v. delivery of cyclosporine suffers from similar problems as the currently practiced i.v. delivery of taxol, i.e., anaphylactic and allergic reactions believed to be due to the Cremaphor, the delivery vehicle employed for the i.v. formulation. In addition, the intravenous delivery of drug (e.g., cyclosporine) encapsulated as described here avoids dangerous peak blood levels immediately following administration of drug. For example, a comparison of currently available formulations for cyclosporine with the above-described encapsulated form of cyclosporine showed a five-fold decrease in peak blood levels of cyclosporine immediately following injection.

In order to avoid problems associated with the Cremaphor, cyclosporine contained within polymeric shells as described above may be delivered by i.v. injection. It may be dissolved in a biocompatible oil or a number of other solvents following which it may be dispersed into polymeric shells by sonication as described above. In addition, an important advantage to delivery cyclosporine (or other immunosuppressive agent) in polymeric shells has the advantage of local targeting due to uptake of the injected material by the RES system in the liver. This may, to some extent, avoid systemic toxicity and reduce effective dosages due to local targeting.

Example 8

Antibody Targeting of Polymeric Shells

The nature of the polymeric shells of the invention allows for the attachment of monoclonal or polyclonal antibodies to the polymeric shell, or the incorporation of antibodies into the polymeric shell. Antibodies can be incorporated into the polymeric shell as the polymeric microcapsule shell is being formed, or antibodies can be attached to the polymeric shell after preparation thereof. Standard protein immobilization techniques can be used for this purpose. For example, with protein microcapsules prepared from a protein such as albumin, a large number of amino groups on the albumin lysine residues are available for attachment of suitably modified antibodies. As an example, antitumor agents can be delivered to a tumor by incorporating antibodies against the tumor into the polymeric shell as it is being formed, or antibodies against the tumor can be attached to the polymeric shell after preparation thereof. As another example, gene products can be delivered to specific cells (e.g., hepatocytes or certain stem cells in the bone marrow) by incorporating antibodies against receptors on the target cells into the polymeric shell as it is being formed, or antibodies against receptors on the target cells can be attached to the polymeric shell after preparation thereof. In addition, monoclonal antibodies against nuclear receptors can be used to target the encapsulated product to the nucleus of certain cell types.

Example 9

Polymeric Shells as Carriers for Polynucleotide Constructs, Enzymes and Vaccines

As gene therapy becomes more widely accepted as a viable therapeutic option (at the present time, over 40 human gene transfer proposals have been approved by NIH and/or FDA review boards), one of the barriers to overcome in implementing this therapeutic approach is the reluctance to use viral vectors for the incorporation of genetic material into the genome of a human cell. Viruses are inherently toxic. Thus, the risks entailed in the use of viral vectors in gene therapy, especially for the treatment of non-lethal, non-genetic diseases, are unacceptable. Unfortunately, plasmids transferred without the use of a viral vector are usually not incorporated into the genome of the target cell. In addition, as with conventional drugs, such plasmids have a finite half life in the body. Thus, a general limitation to the implementation of gene therapy (as well as antisense therapy, which is a reverse form of gene therapy, where a

nucleic acid or oligonucleotide is introduced to inhibit gene expression) has been the inability to effectively deliver nucleic acids or oligonucleotides which are too large to permeate the cell membrane.

The encapsulation of DNA, RNA, plasmids, oligonucleotides, enzymes, and the like, into protein microcapsule shells as described herein can facilitate their targeted delivery to the liver, lung, spleen, lymph and bone marrow. Thus, in accordance with the present invention, such biologics can be delivered to intracellular locations without the attendant risk associated with the use of viral vectors. This type of formulation facilitates the non-specific uptake or endocytosis of the polymeric shells directly from the blood stream to the cells of the RES, into muscle cells by intramuscular injection, or by direct injection into tumors. In addition, monoclonal antibodies against nuclear receptors can be used to target the encapsulated product to the nucleus of certain cell types.

Diseases that can be targeted by such constructs include diabetes, hepatitis, hemophilia, cystic fibrosis, multiple sclerosis, cancers in general, flu, AIDS, and the like. For example, the gene for insulin-like growth factor (IGF-1) can be encapsulated into protein shells for delivery for the treatment of diabetic peripheral neuropathy and cachexia. Genes encoding Factor IX and Factor VIII (useful for the treatment of hemophilia) can be targeted to the liver by encapsulation into protein microcapsule shells of the present invention. Similarly, the gene for the low density lipoprotein (LDL) receptor can be targeted to the liver for treatment of atherosclerosis by encapsulation into protein microcapsule shells of the present invention.

Other genes useful in the practice of the present invention are genes which re-stimulate the body's immune response against cancer cells. For example, antigens such as HLA-B7, encoded by DNA contained in a plasmid, can be incorporated into a protein shell of the present invention for injection directly into a tumor (such as a skin cancer). Once in the tumor, the antigen will recruit to the tumor specific cells which elevate the level of cytokines (e.g., IL-2) that render the tumor a target for immune system attack.

As another example, plasmids containing portions of the adeno-associated virus genome are contemplated for encapsulation into protein microcapsule shells of the present invention. In addition, protein microcapsule shells of the present invention can be used to deliver therapeutic genes to CD8+ T cells, for adoptive immunotherapy against a variety of tumors and infectious diseases.

Protein shells of the present invention can also be used as a delivery system to fight infectious diseases via the targeted delivery of an antisense nucleotide, for example, against the hepatitis B virus. An example of such an antisense oligonucleotide is a 21-mer phosphorothioate against the polyadenylation signal of the hepatitis B virus.

Protein shells of the present invention can also be used for the delivery of the cystic fibrosis transmembrane regulator (CFTR) gene. Humans lacking this gene develop cystic fibrosis, which can be treated by nebulizing protein microcapsule shells of the present invention containing the CFTR gene, and inhaling directly into the lungs.

Enzymes can also be delivered using the protein shells of the present invention. For example, the enzyme, DNAse, can be encapsulated and delivered to the lung. Similarly, ribozymes can be encapsulated and targeted to virus envelop proteins or virus infected cells by attaching suitable antibodies to the exterior of the polymeric shell. Vaccines can also be encapsulated into polymeric microcapsules of the present invention and used for subcutaneous, intramuscular or intravenous delivery.

Example 10

Localized Treatment of Brain Tumors and Tumors Within the Peritoneum

Delivering chemotherapeutic agents locally to a tumor is an effective method for long term exposure to the drug while minimizing dose limiting side effects. The biocompatible materials discussed above may also be employed in several physical forms such as gels, crosslinked or uncrosslinked to provide matrices from which the pharmacologically active ingredient, for example paclitaxel, may be released by diffusion and/or degradation of the matrix. Capxol may be dispersed within a matrix of the biocompatible material to provide a sustained release formulation of paclitaxel for the treatment of brain tumors and tumors within the peritoneal cavity (ovarian cancer and metastatic diseases). Temperature sensitive materials may also be utilized as the dispersing matrix for the invention formulation. Thus for example, the Capxol may be injected in a liquid formulation of the temperature sensitive materials (e.g., copolymers of polyacrylamides or copolymers of polyalkylene glycols and polylactide/glycolides and the like) which gel at the tumor site and provide slow release of Capxol. The Capxol formulation may be dispersed into a matrix of the above mentioned biocompatible polymers to provide a controlled release formulation of paclitaxel, which through the properties of the Capxol formulation (albumin associated with paclitaxel) results in lower toxicity to brain tissue as well as lower systemic toxicity as discussed below. This combination of Capxol or other chemotherapeutic agents formulated similar to Capxol together with a biocompatible polymer matrix may be useful for the controlled local delivery of chemotherapeutic agents for treating solid tumors in the brain and peritoneum (ovarian cancer) and in local applications to other solid tumors. These combination formulations are not limited to the use of paclitaxel and may be utilized with a wide variety of pharmacologically active ingredients including antiinfectives, immunosuppressives and other chemotherapeutics and the like.

Example 11

Stability of Capxol™ Following Reconstitution

Lyophilized Capxol in glass vials was reconstituted with sterile normal saline to concentrations of 1, 5, 10, and 15 mg/ml. and stored at room temperature and under refrigerated conditions. The suspensions was found to be homogeneous for at least three days under these conditions. Particle size measurements performed at several time points indicated no change in size distribution. No precipitation was seen under these conditions. This stability is unexpected and overcomes problems associated with Taxol, which precipitates in within about 24 hours after reconstitution at the recommended concentrations of 0.6–1.2 mg/ml.

In addition, reconstituted Capxol was stable in presence of different polymeric tubing materials such as teflon, silastic, polyethylene, tygon, and other standard infusion tubing materials. This is a major advantage over Taxol which is limited to polyethylene infusion sets and glass infusion bottles.

Example 12

Unit Dosage Forms for Capxol™

Capxol is prepared as a lyophilized powder in vials of suitable size. Thus a desired dosage can be filled in a suitable

container ad lyophilized to obtain a powder containing essentially albumin and paclitaxel in the desired quantity. Such containers are then reconstituted with sterile normal saline or other aqueous diluent to the appropriate volume at the point of use to obtain a homogeneous suspension of paclitaxel in the diluent. This reconstituted solution can be directly administered to a patient either by injection or infusion with standard i.v. infusion sets.

Example 13

Study of Myelosuppression in Rats With Capxol™ and TAXOL® Following a Single Intravenous Administration

Myelosuppression and other hemopoietic effects have been reported as adverse events after treatment with TAXOL. This study was designed to compare the effects of Capxol with TAXOL in rats after a single intravenous injection. The effects of both the Capxol and TAXOL carrier vehicles were also tested. Both Capxol and TAXOL were tested at a dose of 5 mg/kg paclitaxel while the carrier vehicle were tested individually at the respective concentrations used to suspend 5 mg/kg of paclitaxel. Therefore, 766 mg/kg of TAXOL vehicle and 50 mg/kg of Capxol vehicle was administered for these treatments. Changes in body weight and white blood cell counts were used to evaluate the hemopoietic effects.

Capxol produced significantly less ($P<0.05$) myelosuppression than TAXOL as determined by white cell counts at days 1 and 7 and a highly significant ($P<0.01$) reduction in white cell counts at day 10. Capxol also showed significantly less decreases in weight at days 1 and 10 than TAXOL. The TAXOL vehicle decreased WBCs for days 1 and 3 ($P<0.01$) when compared to the Capxol vehicle and also significantly decreased WBCs on day 1 when compared to Capxol ($P<0.05$). Significant decreases in body weights ($P<0.05$) were also observed for the TAXOL vehicle when compared to both Capxol and its vehicle. White cell counts were back to normal by day 7 for the Capxol treated animals but returned to normal only by day 14 for the TAXOL group. Results are presented in Table 2.

TABLE 2

Group	Dose (mg/kg)	# of Animals (n)	Observation
Capxol	5	4	Significantly less myelosuppression and weight loss than with TAXOL
TAXOL	5	4	Significantly greater myelosuppression than Capxol
TAXOL Vehicle	766	2	Decrease in WBCs for day 1 and 3 compared to Capxol vehicle. Significant decrease in WBC on day 1 compared to Capxol
Capxol Vehicle	50	2	No effect on WBC count

It is very surprising that when Capxol and Taxol are administered to rats at equivalent doses of paclitaxel, a much higher degree of myelosuppression results for the Taxol group compared to the Capxol group. This can result in lower incidences of infections and fever episodes (e.g., febrile neutropenia). It can also reduce the cycle time in between treatments which is currently 21 days. With the use of Capxol, this cycle time may be reduced to 2 weeks or less allowing for more effective treatment for cancers. Thus the use of Capxol may provide substantial advantage over Taxol.

Example 14

Determination of the LD_{50} in Mice for Capxol™ and TAXOL® Following a Single Intravenous Administration

The LD_{50} of Capxol, TAXOL and their carrier vehicles was compared following a single intravenous administration. A total of 48 CD1 mice were used. Paclitaxel doses of 30, 103, 367, 548, and 822 mg/kg were tested for Capxol and doses of 4, 6, 9, 13.4, and 20.1 mg/kg paclitaxel for TAXOL. The dose for human albumin, the vehicle for Capxol, was only tested at 4.94 g/kg (corresponds to a dose of 548 mg/mL Capxol) because human albumin is not considered toxic to humans. The doses tested for the TAXOL vehicle (*Cremophor EL®*) were 1.5, 1.9, 2.8, and 3.4 mL/kg which correspond to doses of 9, 11.3, 16.6, and 20.1 mg/kg of paclitaxel, respectively. Three to four mice were dosed with each concentration.

The results indicated that paclitaxel administered in Capxol is less toxic than TAXOL or the TAXOL vehicle administered alone. The LD_{50} and LD_{10} for Capxol were 447.4 and 371.5 mg/kg of paclitaxel, 7.53 and 5.13 mg/kg of paclitaxel in TAXOL, and 1325 and 794 mg/kg of the TAXOL vehicle, (corresponds to a dose of 15.06 and 9.06 mg/kg TAXOL). In this study, the LD_{50} for Capxol was 59 times greater than TAXOL and 29 times greater than the TAXOL vehicle alone. The LD_{50} for paclitaxel in Capxol was 72 times greater than paclitaxel in TAXOL. Review of all the data in this study suggests that the TAXOL vehicle is responsible for much of the toxicity of TAXOL. It was seen that the mice receiving TAXOL and TAXOL vehicle showed classic signs of severe hypersensitivity indicated by bright pink skin coloration shortly after administration. No such reaction was seen for the Capxol and Capxol vehicle groups. Results are presented in Table 3.

TABLE 3

	Single Intravenous Administration					
Group	Dose (mg/kg	# of Animal	# of Deaths	%	LD_{50} (mg/kg	MTD or LD_{10}
Capxol	822	3	3	0	447.4	371.5
	548	4	4	0		
	367	3	0	100		
	103	3	0	100		
	30	3	0	100		
TAXOL	20.1	4	4	0	7.53	5.13
	13.4	4	4	0		
	9	3	2	33		
	6	4	1	75		
	4	3	0	100		

These high doses of Capxol were administered as bolus injections and represent the equivalent of approximately 80–2000 mg/m^2 dose in humans. The LD10 or maximum tolerated dose of Capxol in this study is equivalent to approximately 1000 mg/m^2 in humans. This is significantly higher than the approved human dose of 175 mg/m^2 for TAXOL.

To our surprise, it was found that the vehicle, Cremophor/Ethanol alone caused severe hypersensitivity reactions and death in several dose groups of mice. The LD50 data for the TAXOL vehicle alone shows that it is considerably more toxic than Capxol and significantly contributes to the toxicity of TAXOL. It has been unclear in the literature, the cause of hypersensitivity, however, based on these data, we believe that HSR's can be attributed to the Taxol vehicle.

Example 15

Determination of the LD_{50} in Mice of Capxol™ and TAXOL® Following Multiple Intravenous Administrations

The LD_{50} of Capxol and TAXOL was compared following multiple intravenous administrations. A total of 32 CD1 mice were used. Capxol with paclitaxel doses of 30, 69, and 103 mg/kg were administered daily for five consecutive days. TAXOL with paclitaxel doses of 4, 6, 9, 13.4, and 20.1 mg/kg was administered daily for 5 consecutive days. Four mice were dosed with each concentration. Results are presented in Table 4.

TABLE 4

	Multiple Intravenous Administrations					
Group	Dose (mg/kg	# of Animal	# of Deaths	%	LD_{50} (mg/kg	MTD or LD_{10}
Capxol	103	4	4	0	76.	64.
	69	4	1	75		
	30	4	0	10		
TAXOL	20.1	4	4	0	8.0	4.3
	13.4	4	4	0		
	9	4	2	50		
	6	4	1	75		
	4	4	0	10		

The results indicated that Capxol is less toxic than TAXOL. The LD_{50} and LD_{10} of Capxol were 76.2 and 64.5 mg/kg of paclitaxel, respectively, compared to 8.07 and 4.3 mg/kg of paclitaxel in TAXOL, respectively. In this study, the LD_{50} for Capxol was 9.4 times higher than for TAXOL. The LD_{10} for Capxol was 15 times higher for Capxol than for TAXOL. The results of this study suggests that the Capxol is less toxic than TAXOL when administered in multiple doses at daily intervals.

Example 16

Toxicity and Efficacy of Two Formulation of Capxol and TAXOL

A study was performed to determine the efficacy of Capxol, TAXOL, and the Capxol vehicle in female athymic NCr-nu mice implanted with MX-1 human mammary tumor fragments.

Groups of 5 mice each were given intravenous injections of Capxol formulation VR-3 or VR-4 at doses of 13.4, 20, 30, 45 mg/kg/day for 5 days. Groups of 5 mice were also each given intravenous injections of TAXOL at doses of 13.4, 20 and 30 mg/kg/day for five days. A control group of ten mice was treated with an intravenous injection of Capxol vehicle control (Human Albumin, 600 mg/kg/day) for 5 days. Evaluation parameters were the number of complete tumor regressions, the mean duration of complete regression, tumor-free survivors, and tumor recurrences.

Treatment with Capxol formulation VR-3 resulted in complete tumor regressions at all dose levels. The two highest doses resulted in 100% survival after 103 days. Capxol formulation VR-4 resulted in complete tumor regression in the three highest dose groups, and 60% regressions at 13.4 mg/kg/day. Survival rates after 103 days were somewhat less than with formulation VR-4. Treatment with TAXOL at 30, 20, and 13.4 mg/kg/day resulted in 103 day survival rates of 40%, 20%, and 20% respectively. Treatment with the control vehicle had no effect on tumor growth and the animals were sacrificed after 33 to 47 days. Results are presented in Table 5.

TABLE 5

Dosage	CR/Total			TSF/TR			DCR (days)			Non Specific Deaths/Total		
(mg/kg/day)	VR-3	VR-4	TAX	VR-3	VR-4	TAX	VR-3	VR-4	TAX	VR-3	VR-4	TAX
45	5/5	5/5	NA	5/0	3/2	NA	>88	>73	NA	0/5	0/5	NA
30	5/5	5/5	4/4	5/0	5/0	2/2	>88	>88	>56	0/5	0/5	1/5
20	5/5	5/5	4/4	1/4	2/3	1/3	>51	>47	>57	0/5	0/5	1/5
13.4	4/5	3/5	4/5	0/5	0/5	1/4	10	8	>29	0/5	0/5	0/5

CR = Complete tumor regression;
TFS = Tumor free survivor;
TR = Tumor recurrence;
DCR = days of complete regression

These unexpected and surprising results show an increased efficacy for the two capxol formulation compared to TAXOL. In addition, higher doses of paclitaxel are achieved in the Capxol groups due to lower toxicity of the formulation. These high doses were administered as bolus injections.

Example 17

Blood Kinetics and Tissue Distribution on ^3H-TAXOL™ and Capxol™ Following a Single Intravenous Dose in the Rat

Two studies were performed to compare the pharmacokinetics and tissue distribution of ^3H-paclitaxel formulated in Capxol and TAXOL Injection Concentrate. Fourteen male rats were intravenously injected with 10 mg/kg of ^3H-TAXOL and 10 rats with 4.9 mg/kg. Ten male rats were intravenously injected with 5.1 mg/kg ^3H-Capxol in the above study.

Levels of both total radioactivity and paclitaxel decline bi-phasically in blood of rats following 5 mg/kg IV bolus doses of either ^3H-TAXOL or ^3H-Capxol. However, the

levels of both total radioactivity and paclitaxel are significantly lower following administration of ^{3}H-Capxol following a similar ^{3}H-TAXOL dose. This lower level is more rapidly distributed out of the blood.

The blood HPLC profile shows a similar pattern of metabolism to highly polar metabolite(s) for both ^{3}H Capxol and ^{3}H-TAXOL. However, the rate of metabolism appears significantly slower for ^{3}H-Capxol as 44.2% of blood radioactivity remains as paclitaxel 24 hours post-dose versus 27.7% for ^{3}H-TAXOL. The excretion of radioactivity occurs only minimally in the urine and predominantly in the feces for ^{3}H-Capxol which is similar to reported excretion patterns for ^{3}H-TAXOL. The blood kinetics for total radioactivity and paclitaxel following IV administration of ^{3}H-Capxol or ^{3}H-TAXOL at 5 mg/kg are presented in Table 6.

TABLE 6

Treatment	AUC_{0-24} (μg eq.hr/mL)	Extrapolated C_0 (μg eq/mL)	Observed C_{max} (μg eq/(mL)	Observed T_{max} (hr)	$t_{1/2}\beta$ (hr)
Total Radioactivity	6.1	7.6	4.2	0.03	19.0
^{3}H-Capxol	10.2	19.7	13.5	0.03	19.7
^{3}H-TAXOL Paclitaxel	3.7	7.0	4.0	0.03	11.4
3H-Capxol	5.4	17.1	11.8	0.03	
3H-TAXOL					7.2

The tissue radioactivity levels are higher following ^{3}H-Capxol administration than ^{3}H-TAXOL administration for 12 of 14 tissues. The tissue/blood ppm ratios are higher in all tissues for ^{3}H-Capxol dosed animals as the blood levels are lower. This supports the rapid distribution of ^{3}H-Capxol from the blood to the tissues suggested by the blood kinetic data.

^{3}H-Paclitaxel formulated in Capxol shows a similar pharmacokinetic profile to ^{3}H-paclitaxel formulated in TAXOL for Injection concentrate, but tissue/blood ppm ratios and metabolism rates differ significantly. A significantly lower level of total radioactivity for Capxol treated animals than for TAXOL treated animals in the 2 minute post administration blood sample indicates that the ^{3}H-Capxol is more rapidly distributed out of the blood. However, the rate of metabolism appears significantly slower for ^{3}H-Capxol as 44% of blood reactivity remains as paclitaxel at 24 hours post-administration versus 28% for ^{3}H-TAXOL.

This finding for Capxol is surprising and provides a novel formulation to achieve sustained activity of paclitaxel compared to TAXOL. Taken together with local high concentrations, this enhanced activity should result in increased efficacy for the treatment of primary tumors or metastases in organs with high local concentrations.

Tissue distributions are presented in Table 7 below. The data represent the mean and standard deviations of 10 rats in each group (Capxol and TAXOL).

TABLE 7

Radioactive Residues in Tissues of Male Rats, Expressed as ppm following a single intravenous dose of ^{3}H-Capxol and ^{3}H-Taxol at 5 mg/kg

Sample	Capxol Mean ± SD Values		Taxol Mean ± SD Values	
Brain	0.106	0.008	0.145	0.020
Heart	0.368	0.063	0.262	0.037

TABLE 7-continued

Radioactive Residues in Tissues of Male Rats, Expressed as ppm following a single intravenous dose of ^{3}H-Capxol and ^{3}H-Taxol at 5 mg/kg

Sample	Capxol Mean ± SD Values		Taxol Mean ± SD Values	
Lung	1.006	0.140	0.694	0.057
Liver	1.192	0.128	1.37	0.204
Kidney	0.670	0.110	0.473	0.068
Muscle	0.422	0.120	0.386	0.035
GI Tract	0.802	0.274	0.898	0.243
Testes	0.265	0.023	0.326	0.047
Pancreas	0.963	0.357	0.468	0.070
Carcass	0.596	0.070	0.441	0.065
Bone	0.531	0.108	0.297	0.051
Spleen	0.912	0.131	0.493	0.070
Prostate	1.728	0.356	1.10	0.161
Seminal Vesicles	1.142	0.253	1.20	0.237
Blood	0.131	0.010	0.181	0.020
Plasma	0.131	0.012	0.196	0.026

The data show significantly higher levels of accumulation of Capxol in the several organs when compared to Taxol. These organs include prostate, pancreas, kidney, lung, heart, bone, and spleen. Thus Capxol may be more effective than Taxol in the treatment of cancers of these organs at equivalent levels of paclitaxel.

Levels in the prostate tissue are of particular interest in the treatment of prostatic cancer. This surprising and unexpected result has implications for the treatment of prostate cancer. Table 8 below shows the data for individual rats (10 in each group) showing increased accumulation of paclitaxel in the prostate for Capxol as compared to TAXOL. The basis for the localization within the prostate could be a result of the particle size of the formulation (20–400 nm), or the presence the protein albumin in the formulation which may cause localization into the prostatic tissue through specific membrane receptors (gp 60, gp 18, gp 13 and the like). It is also likely that other biocompatible, biodegradable polymers other than albumin may show specificity to certain tissues such as the prostate resulting in high local concentration of paclitaxel in these tissues as a result of the properties described above. Such biocompatible materials are contemplated within the scope of this invention. A preferred embodiment of a composition to achieve high local concentrations of paclitaxel in the prostate is a formulation containing paclitaxel and albumin with a particle size in the range of 20–400 nm, and free of cremophor. This embodiment has also been demonstrated to result in higher level concentrations of paclitaxel in the, pancreas, kidney, lung, heart, bone, and spleen when compared to Taxol at equivalent doses.

TABLE 8

Data for 10 rats in each group
Dose 5 mg/kg paclitaxel

CAPXOL ™	BMS TAXOL ™
1.228	1.13
2.463	1.04
1.904	0.952
1.850	1.42
1.660	1.31
1.246	1.08

TABLE 8-continued

Data for 10 rats in each group Dose 5 mg/kg paclitaxel	
CAPXOL ™	BMS TAXOL ™
1.895	1.03
1.563	0.95
1.798	0.94
1.676	1.18
Mean	Mean
SD	SD

This unexpected localization of paclitaxel to the prostate in the Capxol formulation may be exploited for the delivery of other pharmacologically active agents to the prostate for the treatment of other disease states affecting that organ, e.g., antibiotics in a similar formulation for the treatment of prostatitis (inflammation and infection of the prostate), therapeutic agents effective for the treatment of benign prostatic hypertrophy maybe formulated in a similar fashion to achieve high local delivery. Similarly, the surprising finding that Capxol provides high local concentrations to the heart can be exploited for the treatment of restenosis as well as atherosclerotic disease in coronary vessels. Paclitaxel has been demonstrated to have a therapeutic effect in the prevention of restentosis and atherosclerosis and Capxol thus is an ideal vehicle. Furthermore it has been demonstrated that polymerized albumin preferentially binds to inflamed endothelial vessels possibly through gp60, gp18 and gp13 receptors.

Example 18

Blood Kinetics and Tissue Distribution of Paclitaxel Following Multiple Intravenous Dose Levels of Capxol™ in the Rat

The study using ^{3}H-Capxol was supplemented by treating four additional groups of rats with a single bolus dose of 9.1 mg/kg, 26.4 mg/kg, 116.7 mg/kg, and 148.1 mg/kg of paclitaxel in Capxol. Blood was collected from the tail vein and the AUC_{0-24} was calculated. At 24 hours, blood samples were collected, extracted, and the extract injected on HPLC to determine the level of parent compound in the blood.

The blood kinetics for total radioactivity and paclitaxel following IV administration of ^{3}H-Capxol are presented in Table 9.

TABLE 9

Group/Dose (mg/kg)	AUC_{0-24} (μg eq.hr/mL)	Extrapolated C_0 (μg eq/mL)	Observed C_{max} (μg eq/(mL)	Observed T_{max} (hr)	$t_{1/2\beta}$ (hr)
A/9.1	11.5	10.2	7.19	0.03	22.3
B/26.4	43.5	44.8	29.5	0.03	16.0
C/116.7	248.9	644.6	283.3	0.03	8.48
D/148.1	355.3	1009.8	414.2	0.03	9.34

As the dose of paclitaxel was increased, the area under the curve was proportionally increased. The level of parent compound after 24 hours was increased by a factor of 8.5 (0.04 ppm–0.34 ppm), going from the 9 mg/kg dose to the 148 mg/kg dose.

Example 19

Determination of the Toxicity in Rats of Capxol™ and TAXOL Following a Single Intravenous Administration

The objective of the study was to determine the toxicity of Capxol™ following a single IV administration in male and female rats. Capxol™ was administered to 6 male and 6 female rats at doses of 5, 9, 30, 90 and 120 mg/kg. One half of the animals from each dose group were euthanized and necropsied on Day 8. The remaining animals were necropsied on Day 31. The results of Capxol™-treated animals were compared to the results of normal saline and vehicle control groups as well as to the results of animals treated with 5, 9 and 30 mg/kg TAXOL.

Animals were examined immediately after dosing, 1 hour and 4 hours past administration and once daily thereafter. Blood was collected from each animal for hematological and serum determination prior to euthanasia.

Thirteen deaths occurred during the 30 day observation period. All 12 animals treated with TAXOL at a dose of 30 mg/kg paclitaxel died by day 4. Only one animal treated with Capxol died. The Capxol treated animal received 90 mg/kg paclitaxel and was found dead on day 15. No other animals treated with Capxol died at the 90 kg or 120 mg/kg dose, therefore the death is not thought to be treatment related.

During the first four hours observation period, piloerection and staggering gait were observed in the majority of animals treated with TAXOL, possibly due to the alcohol content of the drug. Piloerection was noted in a few animals treated with Capxol. Animals treated with TAXOL at a dose of 30 mg/kg paclitaxel were observed with piloerection and lethargy and were found dead by day 4. No overt signs of toxicity were observed in Capxol treated animals, except for a few incidences of piloerection at the 90 mg/mL and 120 mg/mL dose levels.

No abnormalities were reported in Capxol treated animals. Gross necropsy results for day 8 and day 31 were normal. Significant dose related changes were seen in the male reproductive organs in animals treated with Capxol. A degeneration and vacuolation of epididymal ductal epithelial cells, often accompanied by multifocal interstitial lymphocytic infiltrate, was observed. There was increasing severe atrophy of seminiferous tubules seen in the testes as the dose of Capxol increased. In the pathologist's opinion, there were significant lesions observed in the male reproductive organs of the animals treated with 9, 30, 90, and 120 mg/kg Capxol. These changes involved diffuse degeneration and necrosis of the testes. These changes were the most prevalent in animals that received higher doses of Capxol. No changes were seen in the testes from untreated control animals, vehicle control animals, or those treated with TAXOL.

This finding is unexpected and has significant therapeutic implications for the treatment of hormone dependent cancers such as prostate cancer. Removal of the testes (orchiectomy) is a therapeutic approach to the treatment of prostate cancer. Capxol represents a novel formulation for the treatment of this disease by achieving high local concentration of paclitaxel at that site, by sustained activity of the active ingredient, by reduction of testicular function and without the toxic cremophor vehicle. Treatment with Capxol thus allows for reduction in levels of testosterone and other androgen hormones.

Cerebral cortical necrosis was seen at the mid dose level of the TAXOL treated animals. This may explain the deaths of the animals treated with even higher doses of TAXOL. No cerebral lesions were seen in animals treated with Capxol.

This lack of cerebral or neurologic toxicity is surprising and has significant implications in both the treatment of brain tumors and the ability to achieve high systemic doses ranging from 5–120 mg/kg in rats (equivalent to 30–700 mg/m2 dose in humans).

To summarize, Capxol was considerably less toxic than TAXOL. No TAXOL animals survived at the doses higher than 9 mg/kg. With the exception of an incidental death at 90 mg/kg Capxol, all animals which received Capxol survived at doses up to and including 120 mg/kg. There was a high dose-related effect of Capxol on the male reproductive organs and a suppression in male body weight. Female rats did not demonstrate any toxic effects from the administration of Capxol at doses up to and including 120 mg/kg. These high doses were administered as bolus injections and represent the equivalent of 30–700 mg/m^2 dose in humans.

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 administration of a taxane to a subject in need thereof, said method comprising systemically administering a dose in the range of about 30 mg/m^2 to about 1000 mg/m^2 of said taxane to said subject in a pharmaceutically acceptable formulation substantially free of cremophor, over an administration time of less than 3 hours, without the use of premedication.

2. The method of claim 1, wherein said dose is at least 135 mg/m^2.

3. The method of claim 1, wherein said pharmaceutically acceptable formulation is administered as a bolus injection.

4. The method of claim 3, wherein said dose is at least 135 mg/m^2.

5. The method of claim 1, wherein said administration period is less than 2 hours.

6. The method of claim 1, wherein said dose is at least 175 mg/m^2.

7. A method for the administration of a taxane to a subject in need thereof, said method comprising systemically administering a dose in the range of about 30 mg/m^2 to about 1000 mg/m^2 of said taxane to said subject in a pharmaceutically acceptable formulation substantially free of cremophor without the use of premedication, with a treatment cycle of no greater than 2 weeks.

8. The method of claim 7, wherein said dose is at least 135 mg/m^2.

9. The method of claim 7, wherein said dose is at least 175 mg/m^2.

10. The method of claim 7, wherein said administration period is less than 2 hours.

11. The method of claim 7, wherein said pharmaceutically acceptable formulation is administered as a bolus injection.

12. The method of claim 11, wherein said dose is at least 135 mg/m^2.

13. The method of claim 11, wherein said dose is at least 175 mg/m^2.

14. The method of claim 11, wherein said dose is at least 250 mg/m^2.

15. A method for reducing the hematologic toxicity of a taxane in a subject undergoing treatment with taxane, said method comprising systemically administering a dose in the range of about 30 mg/m^2 to about 1000 mg/m^2 of said taxane to said subject in a pharmaceutically acceptable formulation, wherein said pharmaceutically acceptable formulation is substantially free of cremophor.

16. The method of claim 15, wherein said pharmaceutically acceptable formulation further comprises albumin.

17. A method for reducing the cerebral or neurologic toxicity of taxane in a subject undergoing treatment with taxane, said method comprising systemically administering a dose in the range of about 30 mg/m^2 to about 1000 mg/m^2 of said taxane to said subject in a pharmaceutically acceptable formulation, wherein said pharmaceutically acceptable formulation is substantially free of cremophor.

18. The method of claim 17, wherein said pharmaceutically acceptable formulation further comprises albumin.

19. A method for treatment of primary tumors in a subject by achieving high local concentration of taxane at the tumor site, said method comprising systemically administering a dose in the range of about 30 mg/m^2 to about 1000 mg/m^2 of said taxane to said subject in a pharmaceutically acceptable formulation substantially free of cremophor.

20. The method of claim 19, wherein said primary tumors are selected from cancers of prostate, testes, lung, kidney, pancreas, bone, spleen, liver or brain.

21. The method of claim 20, wherein said pharmaceutically acceptable formulation further comprises albumin.

22. A pharmaceutically acceptable formulation of taxane for treatment of primary tumors in a subject which achieves high local concentration of said taxane at the tumor site, said formulation being substantially free of cremophor and comprising taxane in a dose in the range of about 30 mg/m^2 to about 1000 mg/m^2.

23. The formulation of claim 22, wherein said primary tumors are selected from cancers of prostate, testes, lung, kidney, pancreas, bone, spleen, liver or brain.

24. The formulation of claim 22 further comprising albumin.

25. A pharmaceutically acceptable formulation of taxane according to claim 22, wherein said formulation shows reduced cerebral or neurologic toxicity.

26. A method for treatment of metastatic tumors in a subject by achieving high local concentration of taxane at the site of metastases, said method comprising systemically administering a dose in the range of about 30 mg/m^2 to about 1000 mg/m^2 of said taxane to said subject in a pharmaceutically acceptable formulation substantially free of cremophor.

27. The method of claim 26, wherein said site of metastases are selected from lung, bone, liver or brain.

28. The method of claim 26, wherein said pharmaceutically acceptable formulation further comprises albumin.

29. A method for treatment of prostatic cancer in a subject by inducing a medical orchiectomy by administering a dose of taxane in the range of about 30 mg/m^2 to about 1000 mg/m^2 to said subject in a pharmaceutically acceptable formulation substantially free of cremophor.

30. The method of claim 29, wherein said pharmaceutically acceptable formulation further comprises albumin.

31. A unit dosage form comprising a vessel containing a sufficient quantity of taxane to allow systemic administration at a dose in the range of about 30 mg/m^2 to about 1000 mg/m^2 over an administration period of less than 3 hours.

32. A unit dosage form according to claim 31, wherein said taxane is administered as a non-aqueous formulation, wherein said non-aqueous formulation is substantially free of cremophor.

33. A unit dosage form according to claim 31, wherein said taxane is administered as a dry powder formulation.

34. A lyophilized taxane-containing formulation characterized by the ability to be reconstituted at concentrations greater than 1.3 mg/ml, and remaining stable for at least 3 days.

35. A taxane-containing formulation suitable for administration using standard intravenous infusion tubing, wherein said taxane-containing formulation has a concentration of greater than 1.3 mg/ml.

36. A taxane-containing formulation suitable for delivery of a dose in the range of about 30 mg/m^2 to about 1000 mg/m^2 with an administration period of less than 3 hrs.

37. A formulation according to claim 36 wherein said dose comprises about 250–300 mg/m^2.

38. A formulation according to claim 31 wherein said dose is delivered in a volume of <200 ml.

39. A pharmaceutically acceptable formulation of taxane comprising in the range of about 30 mg/m^2 to about 1000 mg/m^2 of taxane, wherein said formulation is useful for the reduction of serum testosterone levels in a subject.

40. The method of claim 1, wherein said dose is greater than 80 mg/m^2 and up to 700 mg/m^2.

41. A unit dosage form according to claim 31, wherein said quantity of taxane is sufficient to allow systemic administration of a taxane dose of greater than 80 mg/m^2 and up to 700 mg/m^2 over an administration period of less than 3 hours.

42. A formulation according to claim 36, wherein said dose is greater than 80 mg/m^2 and up to 700 mg/m^2.

43. The method of claim 1 wherein said formulation is free of cremophor.

44. The method of claim 7 wherein said formulation is free of cremophor.

45. The method of claim 15 wherein said formulation is free of cremophor.

46. The method of claim 17 wherein said formulation is free of cremophor.

47. The method of claim 18 wherein said formulation is free of cremophor.

48. The formulation of claim 22, wherein said formulation is free of cremophor.

49. The method of claim 26 wherein said formulation is free of cremopor.

50. The method of claim 29 wherein said formulation is free of cremophor.

51. The unit dosage form of claim 32 wherein said non-aqueous formulation is free of cremophor.

52. The method of claim 1, wherein said administration period is less than 1 hour.

53. The method of claim 7, wherein said administration period is less than 1 hour.

54. The method of claim 1, wherein said dose is at least 250 mg/m^2.

55. The method of claim 7, wherein said dose is at least 250 mg/m^2.

56. The method of claim 1, wherein said formulation is free of agents which aid recovery from hematologic toxicity of taxane.

57. The method of claim 7, wherein said formulation is free of agents which aid recovery from hematologic toxicity of taxane.

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EXHIBIT 3

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(71) Applicant *(for all designated States except US)*: **AMERICAN BIOSCIENCE, INC.** [US/US]; Suite 110, 2730 Wilshire Boulevard, Santa Monica, CA 90403 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **DESAI, Neil, P.** [IN/US]; 3633 Purdue Avenue, Los Angeles, CA 90066 (US). **TAO, Chunlin** [CN/US]; 221 South Doheny Drive #206, Beverly Hills, CA 90211 (US). **YANG, Andrew** [US/US]; 4309 Rio Hondo Avenue, Rosemead, CA 91770 (US). **LOUIE, Leslie** [US/US]; 1608 Via Palermo, Montebello, CA 90640 (US). **YAO, Zhiwen** [CN/US]; 11121 Garfield Avenue, Culver City, CA 90230 (US). **SOON-SHIONG, Patrick** [US/US]; 12307 Dorothy Street, Los Angeles, CA 90049 (US). **MAGDASSI, Shlomo** [IL/IL]; Hanerd Street 36, Jerusalem (IL).

(74) Agent: **REITER, Stephen, E.**; Gray Cary Ware & Freidenrich, LLP, Suite 1600, 4365 Executive Drive, San Diego, CA 92121 (US).

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(54) Title: PROTEIN STABILIZED PHARMACOLOGICALLY ACTIVE AGENTS, METHODS FOR THE PREPARATION THEREOF AND METHODS FOR THE USE THEREOF

(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 election 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.

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

FIELD OF THE INVENTION

The present invention relates to methods for the production of particulate vehicles for the intravenous administration of pharmacologically active agents, as well as novel compositions produced thereby. In a particular aspect, the invention relates to methods for the *in vivo* delivery of substantially water insoluble pharmacologically active agents (e.g., the anticancer drug paclitaxel, the active ingredient of Taxol™). In another aspect, dispersible colloidal systems containing water insoluble pharmacologically active agents are provided. The suspended particles are encased in a polymeric shell formulated from a biocompatible polymer, and have a diameter of less than about 1 micron. Invention colloidal systems can be prepared without the use of conventional surfactant or any polymeric core matrix. In a presently preferred aspect of the invention, there is provided a method for preparation of extremely small particles which can be sterile-filtered. The polymeric shell contains particles of pharmacologically active agent, and optionally a biocompatible dispersing agent in which pharmacologically active agent can be either dissolved or suspended. Thus, the invention provides a drug delivery system in either liquid form or in the form of a redispersible powder. Either form provides both immediately bioavailable drug molecules (i.e., drug molecules which are molecularly bound to a protein), and pure drug particles coated with a protein.

BACKGROUND OF THE INVENTION

Intravenous drug delivery permits rapid and direct equilibration with the blood stream which carries the medication to the rest of the body. To avoid the peak serum

levels which are achieved within a short time after intravascular injection, administration of drugs carried within stable carriers would allow gradual release of the drugs inside the intravascular compartment following a bolus intravenous injection of the therapeutic nanoparticles.

Injectable controlled-release nanoparticles can provide a pre-programmed duration of action, ranging from days to weeks to months from a single injection. They also can offer several profound advantages over conventionally administered medicaments, including automatic assured patient compliance with the dose regimen, as well as drug targeting to specific tissues or organs (Tice and Gilley, *Journal of Controlled Release* 2:343-352 (1985)).

Microparticles and foreign bodies present in the blood are generally cleared from the circulation by the "blood filtering organs", namely the spleen, lungs and liver. The particulate matter contained in normal whole blood comprises red blood cells (typically 8 microns in diameter), white blood cells (typically 6-8 microns in diameter), and platelets (typically 1-3 microns in diameter). The microcirculation in most organs and tissues allows the free passage of these blood cells. When microthrombii (blood clots) of size greater than 10-15 microns are present in circulation, a risk of infarction or blockage of the capillaries results, leading to ischemia or oxygen deprivation and possible tissue death. Injection into the circulation of particles greater than 10-15 microns in diameter, therefore, must be avoided. A suspension of particles less than 7-8 microns is, however, relatively safe and has been used for the delivery of pharmacologically active agents in the form of liposomes and emulsions, nutritional agents, and contrast media for imaging applications.

The size of particles and their mode of delivery determines their biological behavior. Strand et al. (in *Microspheres-Biomedical Applications*, ed. A. Rembaum, pp 193-227, CRC Press (1988)) have described the fate of particles to be dependent on their size. Particles in the size range of a few nanometers (nm) to 100 nm enter the lymphatic capillaries following interstitial injection, and phagocytosis may occur within the lymph nodes. After intravenous/intraarterial injection, particles less than about 2

microns will be rapidly cleared from the blood stream by the reticuloendothelial system (RES), also known as the mononuclear phagocyte system (MPS). Particles larger than about 7 microns will, after intravenous injection, be trapped in the lung capillaries. After intraarterial injection, particles are trapped in the first capillary bed reached. Inhaled particles are trapped by the alveolar macrophages.

Pharmaceuticals that are water-insoluble or poorly water-soluble and sensitive to acid environments in the stomach cannot be conventionally administered (e.g., by intravenous injection or oral administration). The parenteral administration of such pharmaceuticals has been achieved by emulsification of the oil solubilized drug with an aqueous liquid (such as normal saline) in the presence of surfactants or emulsion stabilizers to produce stable microemulsions. These emulsions may be injected intravenously, provided the components of the emulsion are pharmacologically inert. US Patent No. 4,073,943 describes the administration of water-insoluble pharmacologically active agents dissolved in oils and emulsified with water in the presence of surfactants such as egg phosphatides, pluronics (copolymers of polypropylene glycol and polyethylene glycol), polyglycerol oleate, etc. PCT International Publication No. WO85/00011 describes pharmaceutical microdroplets of an anaesthetic coated with a phospholipid such as dimyristoyl phosphatidylcholine having suitable dimensions for intradermal or intravenous injection.

An example of a water-insoluble drug is paclitaxel, a natural product first isolated from the Pacific Yew tree, *Taxus brevifolia*, by Wani et al. (*J. Am. Chem. Soc.* 93:2325 (1971)). Among the antimitotic agents, paclitaxel, which contains a diterpene carbon skeleton, exhibits a unique mode of action on microtubule proteins responsible for the formation of the mitotic spindle. In contrast with other antimitotic agents such as vinblastine or colchicine, which prevent the assembly of tubulin, paclitaxel is the only plant product known to inhibit the depolymerization process of tubulin, thus preventing the cell replication process.

Paclitaxel, a naturally occurring diterpenoid, has been shown to have significant antineoplastic and anticancer effects in drug-refractory ovarian cancer. Paclitaxel has

shown excellent antitumor activity in a wide variety of tumor models such as the B16 melanoma, L1210 leukemias, MX-1 mammary tumors, and CS-1 colon tumor xenografts. Several recent press releases have termed Taxol™, a paclitaxel formulation, as the new anticancer wonder-drug. Indeed, Taxol™ has recently been approved by the Federal Drug Administration for treatment of ovarian cancer. The poor aqueous solubility of paclitaxel, however, presents a problem for human administration. Indeed, the delivery of drugs that are inherently insoluble or poorly soluble in an aqueous medium can be seriously impaired if oral delivery is not effective. Accordingly, currently used paclitaxel formulations (e.g., Taxol™) require a cremaphor to solubilize the drug. The human clinical dose range is 200-500 mg. This dose is dissolved in a 1:1 solution of ethanol:cremaphor and diluted to one liter of fluid given intravenously. The cremaphor currently used is polyethoxylated castor oil.

In phase I clinical trials, paclitaxel itself did not show excessive toxic effects, but severe allergic reactions were caused by the emulsifiers employed to solubilize the drug to form Taxol™, the conventional formulation of paclitaxel. The current regimen of administration involves treatment of the patient with antihistamines and steroids prior to injection of the drug to reduce the allergic side effects of the cremaphor.

In an effort to improve the water solubility of paclitaxel, several investigators have modified its chemical structure with functional groups that impart enhanced water-solubility. Among them are the sulfonated derivatives (Kingston et al., U.S. Patent 5,059,699 (1991)), and amino acid esters (Mathew et al., *J. Med. Chem.* 35:145-151 (1992)) which show significant biological activity. Modifications to produce a water-soluble derivative facilitate the intravenous delivery of paclitaxel dissolved in an innocuous carrier such as normal saline. Such modifications, however, add to the cost of drug preparation, may induce undesired side-reactions and/or allergic reactions, and/or may decrease the efficiency of the drug.

Protein microspheres have been reported in the literature as carriers of pharmacological or diagnostic agents. Microspheres of albumin have been prepared by either heat denaturation or chemical crosslinking. Heat denatured microspheres are

produced from an emulsified mixture (e.g., albumin, the agent to be incorporated, and a suitable oil) at temperatures between 100°C and 150°C. The microspheres are then washed with a suitable solvent and stored. Leucuta et al. (*International Journal of Pharmaceutics* 41:213-217 (1988)) describe the method of preparation of heat denatured microspheres.

The procedure for preparing chemically crosslinked microspheres involves treating the emulsion with glutaraldehyde to crosslink the protein, followed by washing and storage. Lee et al. (*Science* 213:233-235 (1981)) and U.S. Patent No. 4,671,954 teach this method of preparation.

The above techniques for the preparation of protein microspheres as carriers of pharmacologically active agents, although suitable for the delivery of water-soluble agents, are incapable of entrapping water-insoluble ones. This limitation is inherent in the technique of preparation which relies on crosslinking or heat denaturation of the protein component in the aqueous phase of a water-in-oil emulsion. Any aqueous-soluble agent dissolved in the protein-containing aqueous phase may be entrapped within the resultant crosslinked or heat-denatured protein matrix, but a poorly aqueous-soluble or oil-soluble agent cannot be incorporated into a protein matrix formed by these techniques.

One conventional method for manufacturing drug-containing nanoparticles comprises dissolving polylactic acid (or other biocompatible, water insoluble polymers) in a water-immiscible solvent (such as methylene chloride or other chlorinated, aliphatic, or aromatic solvent), dissolving the pharmaceutically active agent in the polymer solution, adding a surfactant to the oil phase or the aqueous phase, forming an oil-in-water emulsion by suitable means, and evaporating the emulsion slowly under vacuum. If the oil droplets are sufficiently small and stable during evaporation, a suspension of the polymer in water is obtained. Since the drug is initially present in the polymer solution, it is possible to obtain by this method, a composition in which the drug molecules are entrapped within particles composed of a polymeric matrix. The formation of microspheres and nanoparticles by using the solvent evaporation method

has been reported by several researchers (see, for example, Tice and Gilley, in *Journal of Controlled Release* 2:343-352 (1985); Bodmeier and McGinity, in *Int. J. Pharmaceutics* 43:179 (1988); Cavalier et al., in *J. Pharm. Pharmacol.* 38:249 (1985); and D'Souza et al., WO 94/10980) while using various drugs.

Bazile et. al., in *Biomaterials* 13:1093 (1992), and Spenlehauer et al., in Fr Patent 2 660 556, have reported the formation of nanoparticles by using two biocompatible polymers, where one polymer (e.g., polylactide) is dissolved in the organic phase, together with an active component such as a drug, and the other polymer, such as albumin is used as the surface active agent. After emulsification and removal of the solvent, nanoparticles are formed, in which the drug is present inside the polymeric matrix of the polylactide particles.

The properties of the polymer solution from which the polymeric matrix is formed are very important to obtain the proper emulsion in the first stage. For example, polylactide (the polymer commonly used in the preparation of injectable nanoparticles), has a surface activity which causes the rapid adsorption thereof at the dichloromethane-water interface, causing reduced interfacial tension (see, for example, Boury et al., in *Langmuir* 11:1636 (1995)), which in turn improves the emulsification process. In addition, the same researchers found that Bovine Serum Albumin (BSA) interacts with the polylactide, and penetrates into the polylactide monolayer present at the oil-water interface. Therefore, it is expected, based on the above reference, that emulsification during the conventional solvent evaporation method is greatly favored by the presence of the surface active polymer (polylactide) in the nonaqueous organic phase. In fact, the presence of polylactide is not only a sufficient condition, but it is actually necessary for the formation of nanoparticles of suitable size.

Another process which is based on the solvent evaporation method comprises dissolving the drug in a hydrophobic solvent (e.g., toluene or cyclohexane), without any polymer dissolved in the organic solvent, adding a conventional surfactant to the mixture as an emulsifier, forming an oil-in-water emulsion, and then evaporating the solvent to obtain dry particles of the drug (see, for example, Sjostrom et al., in *J.*

Dispersion Science and Technology 15:89-117 (1994)). Upon removal of the nonpolar solvent, precipitation of the drug inside the solvent droplets occurs, and submicron particles are obtained.

It has been found that the size of the particles is mainly controlled by the initial size of the emulsion droplets. In addition, it is interesting to note that the final particle size is reported to decrease with a decrease in the drug concentration in the organic phase. This finding is contrary to the results reported herein, wherein no conventional surfactant is used for the preparation of nanoparticles. In addition, it is noted by the authors of the Sjostrom paper that the drug used, cholesteryl acetate, is surface active in toluene, and hence may be oriented at the oil-water interface; therefore the concentration of drug at the interface is higher, thus increasing the potential for precipitation.

Formation of submicron particles has also been achieved by a precipitation process, as described by Calvo et al. in *J. Pharm. Sci.* 85:530 (1996). The process is based on dissolving 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 particles (216 nm). However, the process is performed at solvent concentrations at which no emulsion is formed.

OBJECTS OF THE INVENTION

Thus it is an object of this invention to deliver pharmacologically active agents (e.g., taxanes (e.g., paclitaxel, docetaxel, and the like), and the like) in unmodified form in a composition that does not cause allergic reactions due to the presence of added emulsifiers 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 microparticles 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 optionally in the absence of any conventional surfactants and/or in the absence of any polymeric core material.

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

BRIEF DESCRIPTION OF THE INVENTION

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 obviates the necessity for administration of substantially water insoluble pharmacologically active agents (e.g., paclitaxel) in an emulsion containing, for example, ethanol and polyethoxylated castor oil, diluted in normal saline (see, for example, Norton et al., in *Abstracts of the 2nd National Cancer Institute Workshop on Taxol & Taxus*, September 23-24, 1992). A disadvantage of such known compositions is their propensity to produce allergic side effects.

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), optionally without the use of any conventional surfactants and/or 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 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 conventional methods such as autoclaving, due to the heat coagulation 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 pharmacologically active agents. Invention compositions comprise substantially water insoluble pharmacologically active agents (as a solid or liquid) coated by an optionally crosslinkable biocompatible polymer, and optionally 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 suspended in a biocompatible aqueous liquid for administration.

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 administration to a mammal. The other portion of the pharmacologically active agent is contained within nanoparticles coated by protein. The nanoparticles containing the pharmacologically active agent are present as a substantially pure active component, without dilution by much, if 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 methods and compositions produced thereby provide for a pharmacologically active agent that is "pre-bound" to a protein (through hydrophobic or ionic interactions) prior to administration.

The present disclosure demonstrates both of the above-described modes of bioavailability for paclitaxel, an anticancer drug capable of binding to human serum albumin (see, for example, Kumar et al., in *Research Communications 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 (i.e., paclitaxel) 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 paclitaxel, as well as other drugs, which enhances the capability of paclitaxel 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 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.

The invention provides, in a particular aspect, a composition of anti-cancer drugs, e.g., paclitaxel, 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., paclitaxel, such compositions cannot 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.

While it is recognized that particles produced according to the invention can be either crystalline, amorphous, or a mixture thereof, it is generally preferred that the drug

be present in the formulation in an amorphous form. This would lead to greater ease of dissolution and absorption, resulting in better bioavailability.

In accordance with another embodiment of the present invention, there are provided various methods of administering a pharmacologically active agent which must be administered in multiple doses over a cycle time which is less than the cycle time of administration of non-invention formulations of the pharmacologically active agent.

The invention further provides various methods of reducing the myelosuppressive effects and/or the neurotoxic effects of a pharmacologically active agent administered to a patient in need thereof.

In accordance with yet another embodiment of the present invention, there are provided methods of administering pharmacologically active agent(s) to a patient having a disease capable of treatment by the pharmacologically active agent(s). Invention methods comprise administering formulations according to the invention containing suitable pharmacologically active agent(s) to the patient. Diseases contemplated for treatment according to the invention include cancers, proliferative diseases, and the like. Administration of invention formulations can be accomplished in a variety of ways, e.g., intravenous or intraarterial, and/or can be without the use of steroids and/or cytokines, and/or can be in combination with a biochemotherapy agent; and/or the single dose levels of pharmacologically active agents can be greater than about 50 mg; and/or the cumulative dose levels of pharmacologically active agents can be greater than about 250 mg/m^2 every 3 weeks.

In accordance with a further embodiment of the present invention, there are provided methods of delivering a pharmacologically active agent to a localized area of a patient for sustained release of the pharmacologically active agent over an extended period of time (e.g., from about 1 day to about 1 year). Invention methods comprise administering to the patient a suitable pharmacologically active agent in the invention formulation, wherein the invention formulation has been dispersed within a matrix of suitable biocompatible material.

In accordance with yet another embodiment of the present invention, there are provided methods of orally administering pharmacologically active agent(s) to a patient in need thereof. Invention methods comprise orally administering an invention formulation of the pharmacologically active agent(s) in combination with intestinal cell efflux inhibitor(s).

In accordance with still another embodiment of the present invention, there are provided methods of administering a combination of suitable pharmacologically active agent(s) to a patient in need thereof. Invention methods comprise administering to the patient 25-75% of the conventionally effective dosage level of each of the suitable pharmacologically active agent(s) in the invention formulation.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 presents the results of intravenous administration of paclitaxel nanoparticles to tumor bearing mice (n=5 in each group), showing a complete regression of tumor in the treatment group (n) compared with a control group receiving saline (l). Virtually uncontrolled tumor growth is seen in the control group. Dose for the treatment group is 20 mg/kg of paclitaxel administered as an intravenous bolus for five consecutive days.

Figure 2 presents the results of intraperitoneal administration of paclitaxel nanoparticles in rats that have developed arthritis in their paws following intradermal injection of collagen. Paw volumes are measured and indicate the severity of the disease. The paw volumes are normalized to 100% at the beginning of treatment. Day 0 represents the initiation of treatment. There are 3 groups - control group receiving saline (n=2, shown as a thin line and labelled in the figure a "non-treatment"); a first treatment group receiving paclitaxel nanoparticles at a dose of 1 mg/kg (n=4, shown as a heavy line and labelled in the figure as "paclitaxel nanoparticles 1.0 mg/kg"), and a second treatment group receiving combination therapy of paclitaxel nanoparticles at a dose of 0.5 mg/kg and prednisone at a dose of 0.2 mg/kg (n=4, shown as a heavy line and labelled in the figure as "prednisone 0.2 mg/kg + paclitaxel nanoparticles 0.5 mg/kg"). The two treatment groups show a dramatic reduction in paw volume with

time, indicating a regression of arthritis, while the control group showed an increase in paw volume over the same period.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided methods for the preparation of substantially water insoluble pharmacologically active agents 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 optionally contains substantially no surfactants,

in a high pressure homogenizer to a predetermined pressure. This predetermined pressure can be in the range of about 100 up to about 100,000 psi, and preferably in the range of about 2,000 up to about 60,000 psi, and can be in a presently preferred range of about 3,000 to about 40,000 psi. In one operational embodiment, such processes can be carried out at a predetermined pressure in the range of about 3,000 psi up to about 30,000 psi. Optionally, the organic and/or aqueous phases are thereafter removed from the mixture after having been subjected to high shear conditions.

Also provided in accordance with the present invention are compositions prepared by the above-described method.

In accordance with a further embodiment of the present invention, there is provided a drug delivery system comprising particles of a solid or liquid, substantially water insoluble pharmacologically active agent, coated with a protein,

wherein said protein coating has free protein associated therewith,

wherein a portion of said pharmacologically active agent is contained within said protein coating and a portion of said pharmacologically active agent is associated with said free protein, and

wherein the average diameter of said particles is no greater than about 1 micron.

The above-described compositions are particularly advantageous as they have been observed to provide a very low toxicity form of a variety of pharmacologically active agents, e.g., the combination of paclitaxel and albumin (as the biocompatible polymer) is a presently preferred combination because of its low toxicity. The combination of paclitaxel and albumin also has the added advantage of being substantially non-myelosuppressive.

In a preferred embodiment, the average diameter of the above-described particles is no greater than about 200 nm. Such particles are particularly advantageous as they can be subjected to sterile filtration, thereby obviating the need for more vigorous treatment to achieve sterilization of solutions containing the desired pharmacologically active agent.

As used herein, the term "*in vivo* delivery" refers to delivery of a pharmacologically active agent by a variety of routes of administration, as are well known to those of skill in the art. Thus, exemplary routes of administration include topical, oral, intraarticular, intracisternal, intraocular, intraventricular, intrathecal, intravenous, intramuscular, intraperitoneal, intradermal/transdermal/subcutaneous, intratracheal/inhalational, rectal (i.e., via suppository), vaginal (i.e., via pessary), intracranial, intraurethral, intrahepatic, intraarterial, intratumoral, mucosal, and the like, as well as suitable combinations of any two or more thereof. Further, administration of the pharmacologically active agent contemplated for use in the present invention can be systemic (i.e., administered to the subject as a whole via any of the above routes) or localized (i.e., administered to the specific location of the particular infirmity of the subject via any of the above routes).

Exemplary means for the systemic administration of pharmacologically active agent(s) are well known to those of skill in the art, and include oral (for example, with a sustained release formulation of the pharmacologically active agent), continuous IV infusion, infusion via bolus injection, infusion through in-dwelling catheters, and any other means which can function to deliver the pharmacologically active agent

systemically to the patient in need thereof, and the like, and suitable combinations of any two or more thereof.

Exemplary means for the localized administration of pharmacologically active agent(s) include catheters, implantable or portable infusion devices, slow release delivery vehicles, and any other means which can function to deliver the pharmacologically active agent to the localized area of the infirmity to be treated, and the like, and suitable combinations of any two or more thereof.

Implantable or portable infusion devices contemplated for use in the present invention are well known to those of skill in the art, and include devices which can deliver precise and controlled amounts of the pharmacologically active agent over extended periods. Typically, these are driven by electromagnetic force, and/or osmotic force, and/or hydrostatic force, and/or gaseous pressure, and/or mechanical force. Commonly, implantable infusion devices are capable of being periodically refilled, and of being able to receive the pharmacologically active agent in solid or liquid form.

Exemplary slow release delivery vehicles include, for example, pharmacologically active agent(s) encapsulated in a colloidal dispersion system or in a polymer stabilized system. Useful colloidal dispersion systems include nanocapsules, microspheres, beads, lipid-based systems (including oil-in-water emulsions, micelles, mixed micelles, liposomes, and the like), and the like. The colloidal system presently preferred is a liposome or microsphere. Liposomes are artificial membrane vesicles which are useful as slow release delivery vehicles when injected or implanted. Some examples of lipid-polymer conjugates and liposomes are disclosed in U.S. Patent No., 5,631,018, which is incorporated herein by reference in its entirety. Other examples of slow release delivery vehicles are biodegradable hydrogel matrices (U.S. Patent No. 5,041, 292), dendritic polymer conjugates (U.S. Patent No. 5,714,166), and multivesicular liposomes (Depofoam®, Depotech, San Diego, CA) (U. S. Patent Nos. 5,723,147 and 5,766,627). One type of microspheres suitable for encapsulating

therapeutic agents for local injection (e.g., into subdermal tissue) is poly(D,L)lactide microspheres, as described in D. Fletcher, *Anesth. Analg.* 84:90-94, 1997.

Besides delivering an effective therapeutic dose to the site of the infirmity and decreasing the chance of systemic toxicity, localized administration also decreases the exposure of the pharmacologically active agent to degradative processes, such as proteolytic degradation and immunological intervention via antigenic and immunogenic responses, as well as to systemic clearance processes, such as sequestration in the liver.

As used herein, the term "biocompatible" describes a substance that does not appreciably alter or affect in any adverse way, the biological system into which it is introduced.

Key differences between the pharmacologically active agents coated by a stabilizing agent (and, optionally, contained in a polymeric shell of the stabilizing agent) according to the invention and protein microspheres of the prior art are in the nature of formation and the final state of the protein after formation of the particle, and its ability to carry poorly aqueous-soluble or substantially aqueous-insoluble agents. In accordance with the present invention, the stabilizing agent (e.g., a protein) can optionally be crosslinked as a result of exposure to high shear conditions in a high pressure homogenizer. High shear is used to disperse a dispersing agent containing dissolved or suspended pharmacologically active agent into an aqueous solution of a biocompatible polymer, optionally bearing sulfhydryl or disulfide groups (e.g., albumin) whereby a coating of stabilizing agent (or, optionally, a shell of crosslinked polymer) is formed around fine droplets of non-aqueous medium. The high shear conditions produce cavitation in the liquid that causes tremendous local heating and results in the formation of superoxide ions that are capable of crosslinking the polymer, for example, by oxidizing the sulfhydryl residues (and/or disrupting existing disulfide bonds) to form new, crosslinking disulfide bonds.

In contrast to the invention process, the prior art method of glutaraldehyde crosslinking is nonspecific and essentially reactive with any nucleophilic group present

in the protein structure (e.g., amines and hydroxyls). Heat denaturation as taught by the prior art significantly and irreversibly alters protein structure. In contrast, disulfide formation contemplated by the present invention does not substantially denature the protein. In addition, particles of substantially water insoluble pharmacologically active agents coated with a stabilizing agent (and/or optionally contained within a shell) differ from crosslinked or heat denatured protein microspheres of the prior art because the coating (and/or polymeric shell) produced by the invention process is relatively thin compared to the diameter of the coated particle. It has been determined (by transmission electron microscopy) that the "shell thickness" of the polymeric coat is approximately 25 nanometers for a coated particle having a diameter of 1 micron (1000 nanometers). In contrast, microspheres of the prior art do not have protein shells, but rather, have protein dispersed throughout the volume of the microsphere.

As used herein, the term "micron" refers to a unit of measure of one one-thousandth of a millimeter.

Thus, in accordance with the present invention, a pharmacologically active agent is dissolved in a suitable solvent. Next, a protein (e.g., human serum albumin, other suitable polymers listed below, and the like) is added (into the aqueous phase) to act as a stabilizing agent for the formation of stable nanodroplets.

Unlike conventional methods for nanoparticle formation, polymer need not be dissolved in the mixture. Polymer can desirably be added to the mixture, however, when additional control over the nanoparticle size is warranted. When used, exemplary polymers include polylactic/polyglycolic acids and copolymers, polyvinyl alcohol, polyvinyl acetate, polyesters, and other synthetic and natural polymers functional to aid in control of the nanoparticle size, and the like, and suitable combinations of any two or more thereof.

Further, unlike conventional methods for nanoparticle formation, surfactant need not be added to the mixture. Surfactant can desirably be added to the mixture, however, when additional control over solvation of the pharmacologically active agent is warranted. When used, exemplary surfactants include sodium lauryl sulfate, lecithin,

Spans, Tweens (e.g., tween 80, and the like), block copolymers (e.g., pluronics (e.g., pluronic F-68, and the like), tetronics, and the like), and other pharmaceutically acceptable surfactants, and suitable combinations of any two or more thereof.

In addition, unlike conventional methods for nanoparticle formation, foam suppressant need not be added to the mixture. Foam suppressant can desirably be added to the mixture, however, when additional control over the suppression of foam in the formation of the nanoparticles is warranted. When used, exemplary foam suppressants include silicones, oils, hydrocarbons, alcohols, other compounds which function to suppress foaming in the formation of the nanoparticles, and the like, and suitable combinations of any two or more thereof.

The order in which these components of the mixture are added to the oil phase and/or the aqueous phase can be varied depending on various conditions, as recognized by those of skill in the art.

Thus, although polymer, and/or surfactant, and/or foam suppressant can optionally be added, the oil phase employed in the preparation of invention compositions typically contains only the pharmacologically active agent dissolved in solvent, and the aqueous phase employed in the preparation of invention compositions commonly contains only the protein dissolved in aqueous medium.

Next, an emulsion is formed by homogenization under high pressure and high shear forces. Such homogenization is conveniently carried out in a high pressure homogenizer, typically operated at pressures in the range of about 100 up to about 100,000 psi, and preferably in the range of about 2,000 up to 60,000 psi, and can be in a presently preferred range of about 3,000 to about 40,000 psi. In one operational embodiment, such processes can be carried out at a predetermined pressure in the range of about 3,000 psi up to about 30,000 psi. In a presently preferred embodiment, such processes are carried out at pressures in the range of about 6,000 up to 25,000 psi, and even as high as 40,000 psi. The resulting emulsion comprises very small nanodroplets of the nonaqueous solvent (containing the dissolved pharmacologically active agent) and very small nanodroplets of the protein stabilizing agent. Acceptable methods of

homogenization include processes imparting high shear and cavitation such as high pressure homogenization, high shear mixers, sonication, high shear impellers, and the like. Processes imparting shear and cavitation forces accomplish high pressure homogenization by using devices such as sonicators, homogenizers, mixers, impellers, and the like (e.g., devices commercially available from such sources as Heat Systems, Microfluidics, Avestin, Stansted, APV, Gaulin, Rannie, Ross, Silverson, Niro, and the like), and suitable combinations of any two or more thereof.

When high pressure homogenization equipment (e.g., a microfluidizer, and the like) is utilized, the product passes through an interaction chamber or a homogenizing valve which channels the product through narrow orifices with tortuous paths (10 μm-2000 μm nominal diameter) which provides high levels of shear in order to break down particle size. Different interaction chambers or homogenizing valves provide different levels of shearing force and thus break down the particle size to different extents. Interaction chambers and homogenizing valves are chosen based on their ability to reduce the particle size. The product can also be extruded under pressure through membranes or other devices having small pores whose size is in the range from about 0.025 micron to about several (e.g., up to about 200) microns.

Finally, the solvent is evaporated under reduced pressure to yield a colloidal system composed of protein coated nanoparticles of pharmacologically active agent and protein. As readily recognized by those of skill in the art, a wide variety of methods of evaporation are suitable for use in the practice of the present invention, including using device(s) selected from rotary evaporators, film evaporators, rising film evaporators, falling film evaporators, agitated film evaporators (e.g., Rototherm), concentrators, evaporator/strippers, multistage evaporators, spray driers, lyophilizers, flash evaporators, freeze driers, or combinations of different types of evaporators such as those available from Buchi, LCI, Artisan, Pope, and Niro, or the like, or suitable combinations of any two or more thereof.

Optionally, the colloidal system produced upon evaporation of the solvent can be ultrafiltered for further concentration or to remove small molecules (e.g., organics,

salts, contaminants, and the like). As readily recognized by those of skill in the art, this ultrafiltration can be accomplished by a variety of methodologies adaptable to the practice of the present invention, e.g., by using ultrafiltration device(s) such as those commercially available from Sartorius, Millipore, Pall, and the like. This ultrafiltration can be conducted prior to, in between, or after the optional filtration(s) identified in the succeeding paragraph, e.g., prior to conventional filtration, in between the stages of prefiltration and sterile filtration or after sterile filtration.

As a further optional step, the colloidal system produced upon evaporation of the solvent can be conventionally filtered and/or sterilized by filtration through sterilizing filter(s) (e.g., sterilizing filters such as membrane filters, track etched filters, depth filters and the like, and suitable combinations of any two or more thereof). Exemplary sterilizing filters are commercially available from Sartorius, Millipore, Gelman, Pall, Nuclepore, and the like. Where prefiltration is desirable, prefilter(s) can be utilized prior to sterile filtration.

In addition, the entire process of manufacture of the product (e.g., the preparation of the mixture, and/or the formation of the emulsion by homogenization, and/or the formation of the colloidal system by evaporation of the solvent, and/or the ultrafiltration, and/or the sterile filtration, as applicable) can be conducted in a batchwise mode or in a continuous mode or by a combination of batch and continuous processes.

Thus, for example, the homogenizer equipment mentioned above (for example, the microfluidizer) can be operated in a number of different ways, e.g., utilizing batch processes, continuous processes or a combination of batch and continuous processes. For example, this homogenizer equipment can be operated in the recycle mode with continuous recycling until the product meets the required particle size, and/or with discrete cycling (i.e., all of the product is processed for a fixed number of cycles (passes)), and/or in a continuous mode with recycle while removing a fixed percentage of the recycled product continuously. In addition,

multiple units of the homogenizer equipment can be connected in series to achieve the desired quality for the product.

Similarly, the evaporator equipment can be operated in batch mode, continuous mode or by a combination of batch and continuous processes. For continuous mode evaporation, the product can be processed once through, or can be recycled continuously through the evaporator until such time as the desired quality of product is attained. For batch mode evaporation, the product may be processed once through the evaporator, provided the desired quality of product is achieved.

Following evaporation of solvent, the liquid suspension may be dried to obtain a powder containing the pharmacologically active agent and protein. The resulting powder can be redispersed at any convenient time into a suitable aqueous medium such as saline, buffered saline, water, buffered aqueous media, solutions of amino acids, solutions of vitamins, solutions of carbohydrates, or the like, as well as combinations of any two or more thereof, to obtain a suspension that can be administered to mammals. Methods contemplated for obtaining this powder include freeze-drying, spray drying, and the like.

In accordance with a specific embodiment of the present invention, there is provided a method for the formation of unusually small submicron particles (nanoparticles), i.e., particles which are less than 200 nanometers in diameter. Such particles are capable of being sterile-filtered before use in the form of a liquid suspension. The ability to sterile-filter the end product of the invention formulation process (i.e., the drug particles) is of great importance since it is impossible to sterilize dispersions which contain high concentrations of protein (e.g., serum albumin) by conventional means such as autoclaving.

In order to obtain sterile-filterable particles (i.e., particles <200 nm), pharmacologically active agent(s) is initially dissolved in a substantially water immiscible organic solvent (e.g., a solvent having less than about 5% solubility in water, such as, for example, chloroform, and other suitable solvents and organic solvents as described below) at high concentration, thereby forming an oil phase containing the

pharmacologically active agent(s). The oil phase employed in the process of the present invention generally contains only the pharmacologically active agent(s) dissolved in solvent.

Next, a water miscible organic solvent (e.g., a solvent having greater than about 10% solubility in water, such as, for example, ethanol) is optionally added to the oil phase at a final concentration in the range of about 1% - 99% v/v, more preferably in the range of about 5% - 25% v/v of the total organic phase. The water miscible organic solvent can be selected from such solvents as ethyl acetate, ethanol, tetrahydrofuran, dioxane, acetonitrile, acetone, dimethyl sulfoxide, dimethyl formamide, methyl pyrrolidinone, and the like, and other suitable solvents and organic media as described below. Alternatively, when water miscible solvent is to be added, the mixture of water immiscible solvent with the water miscible solvent is prepared first, followed by dissolution of the pharmacologically active agent(s) in the mixture.

Next, human serum albumin or any other suitable stabilizing agent as described herein is dissolved in aqueous media. This component acts as a stabilizing agent for the formation of stable nanodroplets. Optionally, a sufficient amount of the first organic solvent (i.e., the substantially water immiscible organic solvent discussed above, e.g.., chloroform) is dissolved in the aqueous phase to bring it close to the saturation concentration. A separate, measured amount of the total organic phase (which now contains the pharmacologically active agent(s), the first organic solvent and optionally the second organic solvent) is added to the saturated aqueous phase, so that the phase fraction of the organic phase is between about 0.1% - 50% v/v, and more preferably between 1% and 15% v/v.

As discussed above, polymer(s) and/or surfactant(s) and/or foam suppressant(s) need not be added to the mixture, although such surfactant(s) and/or foam suppressant(s) can be added when additional control over the nanoparticle size, and/or additional control over solvation of the pharmacologically active agent, and/or over the suppression of foam in the formation of the nanoparticle, respectively, is desirable.

Next, a mixture composed of micro and nanodroplets is formed by homogenization at low shear forces. This can be accomplished in a variety of ways, as can readily be identified by those of skill in the art, employing, for example, a conventional laboratory homogenizer operated in the range of about 2,000 up to about 15,000 rpm. This is followed by homogenization under high pressure (i.e., in the range of about 100 up to about 100,000 psi, and preferably in the range of about 2,000 up to about 60,000 psi, and can be in a presently preferred range of about 3,000 to about 40,000 psi). In one operational embodiment, such high pressure homogenization can be carried out at a predetermined pressure in the range of about 3,000 psi up to about 30,000 psi. The resulting mixture comprises an aqueous protein solution (e.g., human serum albumin), the water insoluble pharmacologically active agent, and the organic solvent(s). Finally, solvent is rapidly evaporated under vacuum to yield a colloidal dispersion system (pharmacologically active agent and protein) in the form of extremely small nanoparticles (i.e., particles in the range of about 10nm - 200 nm diameter), and thus can be sterile-filtered, and optionally conventionally filtered and/or ultra-filtered. The preferred size range of the particles is between about 50 nm - 170 nm, depending on the formulation and operational parameters.

Colloidal systems prepared in accordance with the present invention may be further converted into powder form by removal of the water therefrom, e.g., by lyophilization at a suitable temperature-time profile. As recognized by those of skill in the art, other conventional modes of water removal (e.g., spray drying) can be adapted to the practice of the present invention. The protein (e.g., human serum albumin) itself acts as a cryoprotectant, and the powder is easily reconstituted by addition of water, saline or buffer, without the need to use such conventional cryoprotectants as mannitol, sucrose, glycine, and the like. While not required, it is of course understood that conventional cryoprotectants may be added to invention formulations if so desired.

The coating on the pharmacologically active agent(s) (e.g., the polymeric shell containing solid or liquid cores of pharmacologically active agent(s)) allows for the delivery of high doses of the pharmacologically active agent in relatively small volumes. This minimizes patient discomfort at receiving large volumes of fluid and minimizes

hospital stay. In addition, the walls of the polymeric shell or coating are generally completely degradable *in vivo* by proteolytic enzymes (e.g., when the polymer is a protein), resulting in no side effects from the delivery system as is the case with current formulations.

According to this embodiment of the present invention, particles of substantially water insoluble pharmacologically active agents have a cross-sectional diameter of no greater than about 10 microns. A cross-sectional diameter of less than 5 microns is more preferred, while a cross-sectional diameter of less than 1 micron is presently the most preferred for the intravenous route of administration.

Substantially water insoluble pharmacologically active agents contemplated for use in the practice of the present invention include pharmaceutically active agents, diagnostic agents, agents of nutritional value, and the like. Examples of pharmaceutically active agents include:

analgesics/antipyretics (e.g., aspirin, acetaminophen, ibuprofen, naproxen sodium, buprenorphine hydrochloride, propoxyphene hydrochloride, propoxyphene napsylate, meperidine hydrochloride, hydromorphone hydrochloride, morphine sulfate, oxycodone hydrochloride, codeine phosphate, dihydrocodeine bitartrate, pentazocine hydrochloride, hydrocodone bitartrate, levorphanol tartrate, diflunisal, trolamine salicylate, nalbuphine hydrochloride, mefenamic acid, butorphanol tartrate, choline salicylate, butalbital, phenyltoloxamine citrate, diphenhydramine citrate, methotrimeprazine, cinnamedrine hydrochloride, meprobamate, and the like);

anesthetics (e.g., cyclopropane, enflurane, halothane, isoflurane, methoxyflurane, nitrous oxide, propofol, and the like);

antiasthmatics (e.g., Azelastine, Ketotifen, Traxanox, and the like);

antibiotics (e.g., neomycin, streptomycin, chloramphenicol, cephalosporin, ampicillin, penicillin, tetracycline, and the like);

antidepressants (e.g., nefopam, oxypertine, doxepin hydrochloride, amoxapine, trazodone hydrochloride, amitriptyline hydrochloride, maprotiline hydrochloride, phenelzine sulfate, desipramine hydrochloride, nortriptyline hydrochloride, tranylcypromine sulfate, fluoxetine hydrochloride, doxepin hydrochloride, imipramine hydrochloride, imipramine pamoate, nortriptyline, amitriptyline hydrochloride, isocarboxazid, desipramine hydrochloride, trimipramine maleate, protriptyline hydrochloride, and the like);

antidiabetics (e.g., biguanides, hormones, sulfonylurea derivatives, and the like);

antifungal agents (e.g., griseofulvin, keloconazole, amphotericin B, Nystatin, candicidin, and the like);

antihypertensive agents (e.g., propanolol, propafenone, oxyprenolol, Nifedipine, reserpine, trimethaphan camsylate, phenoxybenzamine hydrochloride, pargyline hydrochloride, deserpidine, diazoxide, guanethidine monosulfate, minoxidil, rescinnamine, sodium nitroprusside, rauwolfia serpentina, alseroxylon, phentolamine mesylate, reserpine, and the like);

anti-inflammatories (e.g., (non-steroidal) indomethacin, naproxen, ibuprofen, ramifenazone, piroxicam, (steroidal) cortisone, dexamethasone, fluazacort, hydrocortisone, prednisolone, prednisone, and the like);

antineoplastics (e.g., adriamycin, cyclophosphamide, actinomycin, bleomycin, duanorubicin, doxorubicin, epirubicin, mitomycin, methotrexate, fluorouracil, carboplatin, carmustine (BCNU), methyl-CCNU, cisplatin, etoposide, interferons, camptothecin and derivatives thereof, phenesterine, taxanes and derivatives thereof (e.g., paclitaxel and derivatives thereof, taxotere and derivatives thereof, and the like), vinblastine, vincristine, tamoxifen, piposulfan, and the like);

antianxiety agents (e.g., lorazepam, buspirone hydrochloride, prazepam, chlordiazepoxide hydrochloride, oxazepam, clorazepate dipotassium, diazepam,

hydroxyzine pamoate, hydroxyzine hydrochloride, alprazolam, droperidol, halazepam, chlormezanone, dantrolene, and the like);

immunosuppressive agents (e.g., cyclosporine, azathioprine, mizoribine, FK506 (tacrolimus), and the like);

antimigraine agents (e.g., ergotamine tartrate, propanolol hydrochloride, isometheptene mucate, dichloralphenazone, and the like);

sedatives/hypnotics (e.g., barbiturates (e.g., pentobarbital, pentobarbital sodium, secobarbital sodium, and the like), benzodiazapines (e.g., flurazepam hydrochloride, triazolam, tomazeparm, midazolam hydrochloride, and the like), and the like);

antianginal agents (e.g., beta-adrenergic blockers, calcium channel blockers (e.g., nifedipine, diltiazem hydrochloride, and the like), nitrates (e.g., nitroglycerin, isosorbide dinitrate, pentaerythritol tetranitrate, erythrityl tetranitrate, and the like), and the like);

antipsychotic agents (e.g., haloperidol, loxapine succinate, loxapine hydrochloride, thioridazine, thioridazine hydrochloride, thiothixene, fluphenazine hydrochloride, fluphenazine decanoate, fluphenazine enanthate, trifluoperazine hydrochloride, chlorpromazine hydrochloride, perphenazine, lithium citrate, prochlorperazine, and the like);

antimanic agents (e.g., lithium carbonate, and the like);

antiarrhythmics (e.g., bretylium tosylate, esmolol hydrochloride, verapamil hydrochloride, amiodarone, encainide hydrochloride, digoxin, digitoxin, mexiletine hydrochloride, disopyramide phosphate, procainamide hydrochloride, quinidine sulfate, quinidine gluconate, quinidine polygalacturonate, flecainide acetate, tocainide hydrochloride, lidocaine hydrochloride, and the like);

antiarthritic agents (e.g., phenylbutazone, sulindac, penicillamine, salsalate, piroxicam, azathioprine, indomethacin, meclofenamate sodium, gold sodium thiomalate, ketoprofen, auranofin, aurothioglucose, tolmetin sodium, and the like);

antigout agents (e.g., colchicine, allopurinol, and the like);

anticoagulants (e.g., heparin, heparin sodium, warfarin sodium, and the like);

thrombolytic agents (e.g., urokinase, streptokinase, altoplase, and the like);

antifibrinolytic agents (e.g., aminocaproic acid, and the like);

hemorheologic agents (e.g., pentoxifylline, and the like);

antiplatelet agents (e.g., aspirin, empirin, ascriptin, and the like);

anticonvulsants (e.g., valproic acid, divalproate sodium, phenytoin, phenytoin sodium, clonazepam, primidone, phenobarbitol, phenobarbitol sodium, carbamazepine, amobarbital sodium, methsuximide, metharbital, mephobarbital, mephenytoin, phensuximide, paramethadione, ethotoin, phenacemide, secobarbitol sodium, clorazepate dipotassium, trimethadione, and the like);

antiparkinson agents (e.g., ethosuximide, and the like);

antihistamines/antipruritics (e.g., hydroxyzine hydrochloride, diphenhydramine hydrochloride, chlorpheniramine maleate, brompheniramine maleate, cyproheptadine hydrochloride, terfenadine, clemastine fumarate, triprolidine hydrochloride, carbinoxamine maleate, diphenylpyraline hydrochloride, phenindamine tartrate, azatadine maleate, tripelennamine hydrochloride, dexchlorpheniramine maleate, methdilazine hydrochloride, trimprazine tartrate, and the like);

agents useful for calcium regulation (e.g., calcitonin, parathyroid hormone, and the like);

antibacterial agents (e.g., amikacin sulfate, aztreonam, chloramphenicol, chloramphenicol palmitate, chloramphenicol sodium succinate, ciprofloxacin

hydrochloride, clindamycin hydrochloride, clindamycin palmitate, clindamycin phosphate, metronidazole, metronidazole hydrochloride, gentamicin sulfate, lincomycin hydrochloride, tobramycin sulfate, vancomycin hydrochloride, polymyxin B sulfate, colistimethate sodium, colistin sulfate, and the like);

antiviral agents (e.g., interferon gamma, zidovudine, amantadine hydrochloride, ribavirin, acyclovir, and the like);

antimicrobials (e.g., cephalosporins (e.g., cefazolin sodium, cephradine, cefaclor, cephapirin sodium, ceftizoxime sodium, cefoperazone sodium, cefotetan disodium, cefutoxime azotil, cefotaxime sodium, cefadroxil monohydrate, ceftazidime, cephalexin, cephalothin sodium, cephalexin hydrochloride monohydrate, cefamandole nafate, cefoxitin sodium, cefonicid sodium, ceforanide, ceftriaxone sodium, ceftazidime, cefadroxil, cephradine, cefuroxime sodium, and the like), penicillins (e.g., ampicillin, amoxicillin, penicillin G benzathine, cyclacillin, ampicillin sodium, penicillin G potassium, penicillin V potassium, piperacillin sodium, oxacillin sodium, bacampicillin hydrochloride, cloxacillin sodium, ticarcillin disodium, azlocillin sodium, carbenicillin indanyl sodium, penicillin G potassium, penicillin G procaine, methicillin sodium, nafcillin sodium, and the like), erythromycins (e.g., erythromycin ethylsuccinate, erythromycin, erythromycin estolate, erythromycin lactobionate, erythromycin siearate, erythromycin ethylsuccinate, and the like), tetracyclines (e.g., tetracycline hydrochloride, doxycycline hyclate, minocycline hydrochloride, and the like), and the like);

anti-infectives (e.g., GM-CSF, and the like);

bronchodialators (e.g., sympathomimetics (e.g., epinephrine hydrochloride, metaproterenol sulfate, terbutaline sulfate, isoetharine, isoetharine mesylate, isoetharine hydrochloride, albuterol sulfate, albuterol, bitolterol, mesylate isoproterenol hydrochloride, terbutaline sulfate, epinephrine bitartrate, metaproterenol sulfate, epinephrine, epinephrine bitartrate, and the like), anticholinergic agents (e.g., ipratropium bromide, and the like), xanthines (e.g.,

aminophylline, dyphylline, metaproterenol sulfate, aminophylline, and the like), mast cell stabilizers (e.g., cromolyn sodium, and the like), inhalant corticosteroids (e.g., flurisolidebeclomethasone dipropionate, beclomethasone dipropionate monohydrate,and the like), salbutamol, beclomethasone dipropionate (BDP), ipratropium bromide, budesonide, ketotifen, salmeterol, xinafoate, terbutaline sulfate, triamcinolone, theophylline, nedocromil sodium, metaproterenol sulfate, albuterol, flunisolide, and the like);

hormones (e.g., androgens (e.g., danazol, testosterone cypionate, fluoxymesterone, ethyltostosterone, testosterone enanihate, methyltestosterone, fluoxymesterone, testosterone cypionate,and the like), estrogens (e.g., estradiol, estropipate, conjugated estrogens, and the like), progestins (e.g., methoxyprogesterone acetate, norethindrone acetate, and the like), corticosteroids (e.g., triamcinolone, betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, dexamethasone acetate, prednisone, methylprednisolone acetate suspension, triamcinolone acetonide, methylprednisolone, prednisolone sodium phosphate methylprednisolone sodium succinate, hydrocortisone sodium succinate, methylprednisolone sodium succinate, triamcinolone hexacatonide, hydrocortisone, hydrocortisone cypionate, prednisolone, fluorocortisone acetate, paramethasone acetate, prednisolone tebulate, prednisolone acetate, prednisolone sodium phosphate, hydrocortisone sodium succinate, and the like), thyroid hormones (e.g., levothyroxine sodium, and the like), and the like;

hypoglycemic agents (e.g., human insulin, purified beef insulin, purified pork insulin, glyburide, chlorpropamide, glipizide, tolbutamide, tolazamide, and the like);

hypolipidemic agents (e.g., clofibrate, dextrothyroxine sodium, probucol, lovastatin, niacin, and the like);

proteins (e.g., DNase, alginase, superoxide dismutase, lipase, and the like);

nucleic acids (e.g., sense or anti-sense nucleic acids encoding any therapeutically useful protein, including any of the proteins described herein, and the like);

agents useful for erythropoiesis stimulation (e.g., erythropoietin, and the like);

antiulcer/antireflux agents (e.g., famotidine, cimetidine, ranitidine hydrochloride, and the like);

antinauseants/antiemetics (e.g., meclizine hydrochloride, nabilone, prochlorperazine, dimenhydrinate, promethazine hydrochloride, thiethylperazine, scopolamine, and the like);

oil-soluble vitamins (e.g., vitamins A, D, E, K, and the like);

as well as other drugs such as mitotane, visadine, halonitrosoureas, anthrocyclines, ellipticine, and the like.

Additional examples of pharmaceutically active agents include those compounds which are substantially water insoluble and which are listed in the "Therapeutic Category and Biological Activity Index" of The Merck Index (12th Ed'n, 1996), the entire relevant contents of which are hereby incorporated by reference.

Examples of diagnostic agents contemplated for use in the practice of the present invention include ultrasound contrast agents, radiocontrast agents (e.g., iodo-octanes, halocarbons, renografin, and the like), magnetic contrast agents (e.g., fluorocarbons, lipid soluble paramagnetic compounds, and the like), as well as other diagnostic agents which cannot readily be delivered without some physical and/or chemical modification to accommodate the substantially water insoluble nature thereof.

Examples of agents of nutritional value contemplated for use in the practice of the present invention include amino acids, sugars, proteins, carbohydrates, fat-soluble vitamins (e.g., vitamins A, D, E, K, and the like) or fat, or combinations of any two or more thereof.

Pharmacologically active agent(s) can be present in a broad range of concentrations in the invention composition, as determined by the end use application of the invention composition. For example, pharmacologically active agent(s) can be present in the invention composition in a range from about 0.0001 % w/v to about 90 % w/v, as measured in the final mixture prior to evaporation and lyophilization. In one operational embodiment, when the pharmacologically active agent is paclitaxel, the concentration of the pharmacologically active agent in the organic solvent(s) (i.e., prior to addition of any other optional components of the mixture) can be in the range from about 0.001 mg/ml to about 1000 mg/ml.

A number of biocompatible polymers may be employed in the practice of the present invention for the formation of the stabilizing agent which coats (and, optionally, for the formation of the polymeric shell which surrounds) the substantially water insoluble pharmacologically active agents. Essentially any polymer, natural or synthetic, optionally bearing sulfhydryl groups or disulfide bonds within its structure, may be utilized for the preparation of a coating (e.g., a disulfide crosslinked shell, and the like) about particles of substantially water insoluble pharmacologically active agents. The optional sulfhydryl groups or disulfide linkages may be preexisting within the polymer structure or they may be introduced by a suitable chemical modification. For example, natural polymers such as proteins, peptides, polynucleic acids, polysaccharides (e.g., starch, cellulose, dextrans, alginates, chitosan, pectin, hyaluronic acid, and the like), proteoglycans, lipoproteins, and so on, are candidates for such modification.

Proteins contemplated for use as stabilizing agents in accordance with the present invention include albumins (which contain 35 cysteine residues), immunoglobulins, caseins, insulins (which contain 6 cysteines), hemoglobins (which contain 6 cysteine residues per $a_2ß_2$ unit), lysozymes (which contain 8 cysteine residues), immunoglobulins, a-2-macroglobulin, fibronectins, vitronectins, fibrinogens, lipases, and the like. Proteins, peptides, enzymes, antibodies and combinations thereof, are general classes of stabilizers contemplated for use in the present invention.

A presently preferred protein for use in the formation of a coating (e.g., a polymeric shell) is albumin. Optionally, proteins such as a-2-macroglobulin, a known opsonin, could be used to enhance uptake of the coated (e.g., shell encased) particles of substantially water insoluble pharmacologically active agents by macrophage-like cells, or to enhance the uptake of the coated (e.g., shell encased) particles into the liver and spleen. Specific antibodies may also be utilized to target the nanoparticles to specific locations.

Similarly, synthetic polypeptides containing cysteine residues are also good candidates for formation of a coating (e.g., a shell) about the substantially water insoluble pharmacologically active agents. These synthetic polypeptides can be chemically unmodified, or, optionally, chemically modified (for example, by the introduction of sulfhydryl and/or disulfide linkages).

Exemplary unmodified synthetic polypeptides contemplated for use in the practice of the present invention are such materials as synthetic polyamino acids (optionally containing cysteine residues and/or disulfide groups), polyvinyl alcohol, polyhydroxyethyl methacrylate, polyacrylic acid, polyethyloxazoline, polyacrylamide, polyvinyl pyrrolidinone, polyalkylene glycols, polylactides, polyglycolides, polycaprolactones, or copolymers thereof, and the like, and suitable combinations of any two or more thereof.

In addition, the unmodified synthetic polypeptides contemplated for use in the practice of the present invention listed above are good candidates for chemical modification (for example, by the introduction of sulfhydryl and/or disulfide linkages) and coating formation (e.g., shell formation, caused, for example, by the crosslinking thereof). Thus, for example, contemplated for use in the practice of the present invention are such materials as 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.

Suitable mixtures of any two or more of the foregoing biocompatible polymers are also contemplated for use in the practice of the present invention.

Biocompatible polymer(s) (i.e., the stabilizing agent) is typically added at a concentration in the range of about 0.001 to about 50 % (w/v), more preferably in the range of about 0.1% to about 25% (w/v), with a presently preferred range of about 0.5% to about 5% (w/v), as measured in the final mixture prior to evaporation and lyophilization.

Suitable solvents utilized in accordance with the present invention include chloroform, methylene chloride, ethyl acetate, ethanol, tetrahydrofuran, dioxane, acetonitrile, acetone, dimethyl sulfoxide, dimethyl formamide, methyl pyrrolidinone, and the like, as well as mixtures of any two or more thereof. Additional solvents contemplated for use in the practice of the present invention include soybean oil, coconut oil, olive oil, safflower oil, cotton seed oil, sesame oil, orange oil, limonene oil, C1-C20 alcohols (e.g., 1-butanol, 2-butanol, 1-pentanol, 3-methyl 1-butanol, and the like), C2-C20 esters (e.g., butyl acetate, isobutyl acetate, isopropyl acetate, n-isopropyl acetate, and the like), C3-C20 ketones, polyethylene glycols, aliphatic hydrocarbons (e.g., heptane, pentane, and the like), aromatic hydrocarbons, halogenated hydrocarbons, and combinations thereof.

Additionally, in the preparation of invention compositions, a wide variety of organic media can also be employed to suspend or dissolve the substantially water insoluble pharmacologically active agent. Organic media contemplated for use in the practice of the present invention include any nonaqueous liquid that is capable of suspending or dissolving the pharmacologically active agent, but does not chemically react with either the polymer employed to produce the shell, or the pharmacologically

active agent itself. Examples include vegetable oils (e.g., soybean oil, olive oil, and the like), coconut oil, safflower oil, cotton seed oil, sesame oil, orange oil, limonene oil, aliphatic, cycloaliphatic, or aromatic hydrocarbons having 4-30 carbon atoms (e.g., n-dodecane, n-decane, n-hexane, cyclohexane, toluene, benzene, and the like), aliphatic or aromatic alcohols having 2-30 carbon atoms (e.g., octanol, and the like), aliphatic or aromatic esters having 2-30 carbon atoms (e.g., ethyl caprylate (octanoate), and the like), alkyl, aryl, or cyclic ethers having 2-30 carbon atoms (e.g., diethyl ether, tetrahydrofuran, and the like), alkyl or aryl halides having 1-30 carbon atoms (and optionally more than one halogen substituent, e.g., CH_3Cl, CH_2Cl_2, CH_2Cl-CH_2Cl, and the like), ketones having 3-30 carbon atoms (e.g., acetone, methyl ethyl ketone, and the like), polyalkylene glycols (e.g., polyethylene glycol, and the like), or combinations of any two or more thereof.

Especially preferred combinations of organic media contemplated for use in the practice of the present invention typically have a boiling point of no greater than about 200°C, and include volatile liquids such as dichloromethane, chloroform, ethyl acetate, benzene, and the like (i.e., solvents that have a high degree of solubility for the pharmacologically active agent, and are soluble in the other organic medium employed), along with a higher molecular weight (less volatile) organic medium. When added to the other organic medium, these volatile additives help to drive the solubility of the pharmacologically active agent into the organic medium. This is desirable since this step is usually time consuming. Following dissolution, the volatile component may be removed by evaporation (optionally under vacuum).

Suitable solvent and/or organic media is typically added at a concentration in the range of about 0.01 % (w/v) to about 50 % (w/v), as measured in the final mixture prior to evaporation and lyophilization.

Particles of pharmacologically active agent(s) associated with a coating (e.g., a polymeric shell), prepared as described above, are delivered as a suspension in a biocompatible aqueous liquid. This liquid may be selected from water, saline, a solution

containing appropriate buffers, a solution containing nutritional agents such as amino acids, sugars, proteins, carbohydrates, vitamins or fat, and the like.

In accordance with a further embodiment of the present invention, there are provided methods of administering a pharmacologically active agent which must be administered in multiple doses over a cycle time which is less than the cycle time of administration of non-invention formulations of the pharmacologically active agent. Invention methods comprise administering the pharmacologically active agent in the invention formulation over a reduced cycle time.

As utilized herein, "cycle time" means the time between administration of consecutive single doses of a pharmacologically active agents which must be administered in multiple doses. Reduced cycle times contemplated for use in the present invention can be described in both absolute and percentage terms. Measured in absolute terms, reduced cycle times are cycle times which have been reduced from the cycle time of the conventional mode of administration of the same dose by any number of days from about 1 day to about 18 days. Measured in percentage terms, reduced cycle times are cycle times which have been reduced from the cycle time of the conventional mode of administration of the same dose by about 5% up to about 85% of the cycle time of the conventional mode of administration of the same dose.

Thus, when the pharmacologically active agent is a taxane (e.g., paclitaxel, docetaxel, and the like), the invention formulation of this taxane can be administered in multiple doses at a reduced cycle time of 1-20 days as required to provide maximum benefit from treatment. The reduced cycle time afforded by the low toxicity of the invention formulations permits more flexibility in treatment than the higher cycle time (i.e., not less than 3 weeks) necessitated by the higher toxicity of conventional formulations of paclitaxel (i.e., Taxol™) and docetaxel (i.e., Taxotere™).

In accordance with another embodiment of the present invention, there are provided methods of reducing the myelosuppressive effects of a pharmacologically active agent administered to a patient in need thereof. Invention methods comprise

administering the pharmacologically active agent in the invention formulation. In one aspect of this embodiment of the invention, the pharmacologically active agent is a taxane (e.g., paclitaxel, docetaxel, and the like).

In accordance with an additional embodiment of the present invention, there are provided methods of reducing the neurotoxicity of a pharmacologically active agent administered to a patient in need thereof. Invention methods comprise administering the pharmacologically active agent in the invention formulation. In one aspect of this embodiment of the invention, the pharmacologically active agent is a taxane (e.g., paclitaxel, docetaxel, and the like).

In accordance with a further embodiment of the present invention, there are provided methods of administering pharmacologically active agent(s) to a patient having a disease capable of treatment by the pharmacologically active agent(s). Invention methods comprise administering suitable pharmacologically agent(s) in the invention formulation to the patient.

Accordingly, when the disease is a proliferative disease (e.g., psoriasis, multiple sclerosis, vascular restinosis, and the like), one aspect of this embodiment comprises administering suitable pharmacologically active agent(s) capable of treating the proliferative disease in the invention formulation to a patient in need thereof. Optionally, the administration is intravenous and/or the formulation is substantially free of surfactant. In one example of this aspect, suitable pharmacologically active agent(s) comprise anti-neoplastic agent(s) (for example, taxane (e.g., paclitaxel, docetaxel, and the like), and the like), or the like.

Similarly, when the disease is a cancer treatable by systemic administration of pharmacologically active agent(s), a further aspect of this embodiment comprises administering suitable pharmacologically active agent(s) capable of treating the cancer in the invention formulation to a patient having the cancer. Administration can be accomplished in a variety of ways, e.g., intravenously. In one example of this aspect, suitable pharmacologically active agent(s) comprise an anti-neoplastic agent (for example, a taxane (e.g., paclitaxel, docetaxel, and the like), and the like), or the like;

and/or the cancer treatable by systemic administration is selected from metastatic breast cancers, malignant melanomas, lung cancers, ovarian cancers, head and neck cancers, prostate cancers, or the like.

In addition, when the disease is a cancer treatable by localized administration of pharmacologically active agent(s), an additional aspect of this embodiment comprises administering suitable pharmacologically active agent(s) capable of treating the cancer in the invention formulation to a patient having the cancer. Administration can be accomplished in a variety of ways, e.g., intraarterially and/or via injection. In an example of this aspect, suitable pharmacologically active agent(s) comprise an anti-neoplastic agent (for example, a taxane (e.g., paclitaxel, docetaxel, and the like), and the like), or the like; and/or the cancer treatable by localized administration is selected from primary and secondary liver tumors (e.g., hepatocellular carcinoma, multifocal hepatoma, and the like), solid tumors with local-regional involvement (e.g., metastatic breast cancer, prostatic cancer, pancreatic cancer, non-small cell lung cancer (squamous cell cancer), colon cancer, renal cancer, intestinal sarcoma, esophageal cancer, melanoma, ependymoma, head and/or neck cancer, and the like), or the like. The dose of suitable pharmacologically active agent administered in accordance with this aspect of the present invention is typically larger than doses administered as part of conventional formulations, and is commonly greater than about 50 mg.

Further, when conventional administration of the suitable pharmacologically active agent(s) requires corticosteroid premedication, an additional aspect of this embodiment comprises administering to the patient the suitable pharmacologically active agent(s) in the invention formulation without the use of any steroid(s). Administration can be accomplished in a variety of ways, e.g., intravenously. In one example of this aspect, the suitable pharmacologically active agent(s) whose conventional administration requires corticosteroid premedication comprises a taxane (e.g., paclitaxel, docetaxel, and the like), or the like; and/or the disease is selected from cancers (e.g., metastatic melanomas, renal cell carcinomas, and the like), or the like.

As a further optional step of this aspect, biochemotherapy agent(s) can be administered to the patient in combination with the suitable pharmacologically active agent(s) in the invention formulation without the use of any steroid(s). These biochemotherapy agent(s) can be administered prior to and/or concurrently with, as well as separately from or jointly with, the suitable pharmacologically active agent(s) in the invention formulation. In one example of this aspect, the biochemotherapy agents are selected from cytokines (e.g., interleukins (e.g., IL-2), interferons, G-CSF, and the like), or the like.

In addition, when conventional administration of a particular dose regimen (e.g., as described by dose level, cycle time, and the like) of the suitable pharmacologically active agent(s) requires further administration of a cytokine, an additional aspect of this embodiment comprises administering to the patient the suitable pharmacologically active agent(s) in the invention formulation without the use of any cytokine.

Particular dose regimens which require further administration of a cytokine can be readily determined by those of skill in the art. Exemplary dose regimens include administration of a suitable pharmacologically active agent (e.g., taxane (for example, paclitaxel, docetaxel, and the like), and the like) at a cumulative dose of greater than about 250 mg/m^2 every 3 weeks, a cumulative dose of greater than about 100-130 mg/m^2 every 1 week, and the like.

In one example of this aspect, the suitable pharmacologically active agent whose conventional administration at a particular dose regimen requires further administration of a cytokine comprises an anti-neoplastic agent (for example, taxane(s) (e.g., paclitaxel, docetaxel, and the like), and the like), or the like; and/or the disease is selected from cancers (e.g., metastatic melanomas, renal cell carcinomas, and the like), or the like.

In accordance with another embodiment of the present invention, there are provided methods of delivering pharmacologically active agent(s) to a localized area of a patient for sustained release of the pharmacologically active agent(s) over an

extended period of time. Invention methods comprise administering to the patient a suitable pharmacologically active agent(s) in the invention formulation, wherein the invention formulation has been dispersed within a matrix of suitable biocompatible material. Administration can be accomplished in a variety of ways, e.g., intravenously. In one aspect of this embodiment of the invention, the suitable pharmacologically active agent is a taxane (e.g., paclitaxel, docetaxel, and the like); the localized areas are selected from sites of brain tumors, sites of tumors within the peritoneal cavity (e.g., ovarian cancer, metastatic disease, and the like), or the like; and the extended period of time is in a range from about 1 day to about 1 year.

Optionally, temperature sensitive materials (e.g., copolymers of polyacrylamides, copolymers of polyalkylene glycols and/or polylactide/glycolides, and the like) which gel at the local site (e.g., localized tumor site, and the like) can be utilized as the dispersing matrix for the invention formulation. In addition, gels could be made of other polysaccharides (e.g., chemically modified hyaluronic acid, and the like) and/or proteins (e.g., albumin, and the like) for controlled release of drugs from nanoparticle formulations.

These matrix-dispersed formulations can be delivered locally by a variety of means of local delivery, as discussed above (e.g., implantation directly into the brain or the peritoneal cavity after surgical removal of the brain tumor or peritoneal-located tumor, respectively, and the like). When temperature sensitive materials are utilized in the formation of this matrix, the invention formulations can be injected in a liquid formulation of the temperature sensitive materials which gels at the tumor site and provides for slow release of the pharmacologically active agent(s).

By manipulating conditions such as the concentration of sodium alginate, the concentration of the invention formulation (e.g., Capxol™, for invention formulations of paclitaxel) in the alginate solution, the crosslink density of the alginate gelled bead, and the size of the implanted beads, the rate of release of the drug can be controlled.

The invention formulations which are dispersed in matrices of the above mentioned biocompatible polymers exhibit a number of potential advantages. First,

these matrix-dispersed formulations can provide a controlled, sustained release formulation of pharmacologically active agent. Second, these matrix-dispersed formulations can provide a locally deliverable formulation of pharmacologically active agent. Third, these matrix-dispersed formulations can result in lower toxicity to the local tissue surrounding the localized treatment site (e.g., the brain tissue, for administration to treat brain tumors) and lower systemic toxicity. Other advantages of these matrix-dispersed formulations are readily apparent to those of skill in the art.

In accordance with yet another embodiment of the present invention, there are provided methods of orally administering pharmacologically active agent(s) to a patient in need thereof. Invention methods comprise orally administering an invention formulation of the pharmacologically active agent(s) in combination with an intestinal cell efflux inhibitor(s).

Intestinal cell efflux inhibitor(s) contemplated for use in the present invention include cyclosporin, FK506 (i.e., tacrolimus), compounds which are effective to inhibit the function of the P-glycoprotein efflux pump (associated with intestinal cells) or other like efflux pumps, and the like, and suitable combinations of any two or more thereof.

Without limiting the scope of this embodiment of the invention, the oral administration of the formulation of the pharmacologically active agent (e.g., taxane) is believed to be facilitated by the oral administration (either in parallel (i.e., concurrent with administration of the agent formulation) or in series (i.e., prior to administration of the agent formulation)) of the intestinal cell efflux inhibitor(s) (e.g., cyclosporin and FK506). The intestinal cell efflux inhibitor(s) is believed to inhibit the function of certain efflux pumps (e.g., the P-glycoprotein efflux pump) on intestinal cell walls. Uninhibited by the intestinal cell efflux inhibitor(s), these efflux pumps would likely promptly pump any absorbed formulation of the pharmacologically active agent (e.g., taxane) out of its associated intestinal cell.

Typically, the intestinal cell efflux inhibitor(s) is contained within an invention formulation. The invention formulation of the pharmacologically active agent(s) and

the invention formulation of intestinal cell efflux inhibitor(s) can be separate formulations (for serial administration), or the same formulation (for parallel administration). Once these formulation(s) have been prepared, they can be administered to a patient suffering from any disease in which treatment with the pharmacologically active agent (for example, taxanes (e.g., paclitaxel, docetaxel, and the like), and the like) shows some benefit, utilizing various dose regimens (e.g., dose amounts, cycle times, and the like) necessary to effect treatment of the disease.

In accordance with still another embodiment of the present invention, there are provided methods of administering a combination of suitable pharmacologically active agent(s) to a patient in need thereof. Invention methods comprise administering to the patient 25-75% of the conventionally effective dosage level of each of the suitable pharmacologically active agent(s) in the invention formulation. The reduced dosage levels promote lower toxicities caused by long term use of the suitable pharmacologically active agent(s), while incorporation of the suitable pharmacologically active agent(s) in the invention formulation promotes a longer half-life for the concentration of the administered agent(s) in the patient and thereby provides similar treatment effects as the conventional dosage level. In one example of this aspect, the combination of suitable pharmacologically agent(s) comprises a taxane (e.g., paclitaxel, docetaxel, and the like), and a steroid, and about 50% of the conventionally effective dosage level of each of the taxane and the steroid is administered as part of the invention formulation.

When a taxane (e.g., paclitaxel, docetaxel, and the like) is contemplated for administration in accordance with the invention methods, the amount administered per single dose typically can vary from about 1 mg/m^2 to about 2000 mg/m^2 or greater, and/or the administration time commonly can vary from about 1 minutes to about 30 minutes, and/or the cycle time between consecutive doses generally can be vary from about 1 day to about 20 days.

Those skilled in the art will recognize that several variations are possible within the scope and spirit of this invention. The organic medium within the polymeric shell

may be varied, a large variety of pharmacologically active agents may be utilized, and a wide range of proteins as well as other natural and synthetic polymers may be used in the formation of the walls of the polymeric shell. Applications are also fairly wide ranging. Other than biomedical applications such as the delivery of drugs, diagnostic agents (in imaging applications), artificial blood and parenteral nutritional agents, the polymeric shell structures of the invention may be incorporated into cosmetic applications such as skin creams or hair care products, in perfumery applications, in pressure sensitive inks, and the like.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1

Preparation of Nanoparticles by High Pressure Homogenization

30 mg paclitaxel is dissolved in 3.0 ml methylene chloride. The solution was added to 27.0 ml of human serum albumin solution (1 % w/v). The mixture was homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification was performed at 9000-18,000 psi while recycling the emulsion for at least 5 cycles. The number of cycles through the homogenizer can vary from about one up to hundreds of cycles, depending on the size of the dispersion desired. The resulting system was transferred into a Rotary evaporator, and methylene chloride was rapidly removed at 40°C, at reduced pressure (30 mm Hg), for 20-30 minutes. The resulting dispersion was translucent, and the typical diameter of the resulting paclitaxel particles was 160-220 (Z-average, Malvern Zetasizer).

The dispersion was further lyophilized for 48 hrs. without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original

dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

Example 2

Preparation of Nanoparticles by Sonication

The purpose of this example is to demonstrate the formation of nanoparticles of paclitaxel by using cavitation and high shear forces during a sonication process. Thus, 20 mg paclitaxel is dissolved in 1.0 ml methylene chloride. The solution is added to 4.0 ml of human serum albumin solution (5% w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I.Q.) in order to form a crude emulsion, and then transferred into a 40 kHz sonicator cell. The sonicator is performed at 60-90% power at 0 degree for 1 min (550 Sonic Dismembrator). The mixture is transferred into a Rotary evaporator, and methylene chloride is rapidly removed at 40°C, at reduced pressure (30 mm Hg), for 20-30 minutes. The typical diameter of the resulting paclitaxel particles was 350-420 nm (Z-average, Malvern Zetasizer).

The dispersion was further lyophilized for 48 hrs. without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

Example 3

Use of Conventional Surfactants and Proteins Results in formation of Large crystals

The following example demonstrates the effect of adding surfactants which are used in the conventional solvent evaporation method. A series of experiments was conducted employing a similar procedure to that described in Example 1, but a surfactant such as Tween 80 (1% to 10%) is added to the organic solvent. It was found that after removal of the methylene chloride, a large number of paclitaxel crystals is obtained having an average size of 1-2 micron, as viewed by light microscopy and under polarized light. The crystals grow within a few hours to form very large needle-like

crystals, with a size in the range of about 5-15 micron. A similar phenomenon is observed with other commonly used surfactants, such as Pluronic F-68, Pluronic F 127, Cremophor EL and Brij 58.

From these results it can be concluded that the conventional solvent evaporation method utilizing conventional surfactants in combination with a protein such as albumin is not suitable for the formation of submicron drug particles (e.g., paclitaxel) without a polymeric core, while using a polar solvent (e.g., methylene chloride).

Example 4

Use of Conventional Surfactants Alone

Results in formation of Large crystals

This example demonstrates that it is not possible to form nanoparticles while using conventional surfactants, without a polymeric core material, with pharmacologically active agents which are soluble in polar, water immiscible solvents (e.g., chloroform).

30 mg paclitaxel is dissolved in 0.55 ml chloroform and 0.05 ml ethanol. The solution is added to 29.4 ml of Tween 80 solution (1 % w/v), which is presaturated with 1% chloroform. The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000-18,000 psi while recycling the emulsion for at least 6 cycles. The number of cycles through the homogenizer can vary from about one up to hundreds of cycles, depending on the size of the dispersion desired. The resulting system was transferred into a Rotary evaporator, and the chloroform was rapidly removed at 40°C, at reduced pressure (30 mm Hg), for 15-30 minutes. The resulting dispersion was opaque, and contained large needle-like crystals of the drug. The initial size of the crystals (observed also by polarized light), was 0.7- 5 micron. Storage of the dispersion for several hours at room temperature led to further increase in crystal size, and ultimately to precipitation.

Example 5

Preparation of Less than 200 nm
Sterile-Filterable Nanoparticles

This example describes the process by which sterile-filterable drug particles can be obtained. Thus, 30 mg paclitaxel is dissolved in 0.55 ml chloroform and 0.05 ml ethanol. The solution is added to 29.4 ml of human serum albumin solution (1 % w/v), which is presaturated with 1% chloroform. The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000-18,000 psi while recycling the emulsion for at least 6 cycles. The number of cycles through the homogenizer can vary from about one up to hundreds of cycles, depending on the size of the dispersion desired. The resulting system is transferred into a Rotary evaporator, and the chloroform is rapidly removed at 40°C, at reduced pressure (30 mm Hg), for 15-30 minutes. The resulting dispersion is translucent, and the typical diameter of the resulting paclitaxel particles is 140-160 nm (Z-average, Malvern Zeta Sizer). The dispersion is filtered through a 0.22 micron filter (Millipore), without any significant change in turbidity, or particle size. HPLC analysis of the paclitaxel content revealed that more than 97% of the paclitaxel was recovered after filtration, thus providing a sterile paclitaxel dispersion.

The sterile dispersion was further lyophilized for 48 hrs. without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

Example 6

Preparation of Less than 200 nm

Sterile-Filterable Nanoparticles

This example describes the process by which sterile-filterable drug particles can be obtained. Thus, 225 mg paclitaxel is dissolved in 2.7 ml chloroform and 0.3 ml ethanol. The solution is added to 97 ml of human serum albumin solution (3 % w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000-18,000 psi while recycling the emulsion for at least 6 cycles. The number of cycles through the homogenizer can vary from about one up to hundreds of cycles, depending on the size of the dispersion desired. The resulting system is transferred into a Rotary evaporator, and the chloroform is rapidly removed at 40°C, at reduced pressure (30 mm Hg), for 15-30 minutes. The resulting dispersion is translucent, and the typical diameter of the resulting paclitaxel particles is 140-160 nm (Z-average, Malvern Zeta Sizer). The dispersion is filtered through a 0.22 micron filter (Sartorius, sartobran 300), without any significant change in turbidity, or particle size. HPLC analysis of the paclitaxel content typically revealed that 70-100% of the paclitaxel could be recovered after filtration, depending on the conditions employed. Thus, a sterile paclitaxel dispersion was obtained.

The sterile dispersion was aseptically filled into sterile glass vials and lyophilized without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

Example 7

Preparation of Less than 200 nm

Sterile-Filterable Nanoparticles - Higher PSI

This example describes the process by which sterile-filterable drug particles can be obtained at higher homogenization pressures than utilized in Example 6. Thus, 225

mg paclitaxel is dissolved in 2.7 ml chloroform and 0.3 ml ethanol. The solution is added to 97 ml of human serum albumin solution (3 % w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Microfluidics). The emulsification is performed at 30,000 psi while recycling the emulsion for at least 6 cycles. The number of cycles through the homogenizer can vary from about one up to hundreds of cycles, depending on the size of the dispersion desired. The resulting system is transferred into a Rotary evaporator, and the chloroform is rapidly removed at 40°C, at reduced pressure (30 mm Hg), for 15-30 minutes. The resulting dispersion is translucent, and the typical diameter of the resulting paclitaxel particles is 120-160 nm (Z-average, Malvern Zeta Sizer). The dispersion is filtered through a 0.22 micron filter (Sartorius, sartobran 300), without any significant change in turbidity, or particle size. HPLC analysis of the paclitaxel content typically revealed that 70-100% of the paclitaxel could be recovered after filtration, depending on the conditions employed. Thus, a sterile paclitaxel dispersion was obtained.

The sterile dispersion was aseptically filled into sterile glass vials and lyophilized without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

Example 8
Effect of Phase Fraction of Organic Solvent on Particle size

The following example demonstrates the importance of having an unusually low phase fraction of the organic solvent in the system.

Thus, a series of experiments was conducted following a similar procedure to that described for Example 5, except the phase fraction of the organic solvent was altered, and the ethanol content maintained at 10% v/v in the organic phase. It was found that increasing the phase fraction led to a significant increase in particle size: at

4% v/v phase fraction (above the saturation concentration, or 5% v/v total chloroform concentration) the resulting particles have a diameter of 250 nm; at 3% v/v phase fraction, the particles have a 200 nm diameter, and at 2% v/v phase fraction, the particles have a 150 nm diameter.

Clearly, only the particles prepared at very low phase fraction could be sterile-filtered.

Example 9

Effect of Drug Concentration on Particle Size

The role of drug concentration in the organic phase is demonstrated in the following example. Two experiments were performed in which the paclitaxel concentration in the organic phase was 50 mg/ml or 75 mg/ml, while all other parameters were the same as described in Example 3. It was found that the low drug concentration yielded particles having a diameter of about 150 nm, while those prepared at the higher drug loading were smaller, i.e., 130-138 nm. When a similar experiment was performed, but with an ethanol concentration in the organic phase of about 50%, a similar trend was observed, i.e., particles were 210 nm and 156 nm in diameter, for 25 mg/ml and 50 mg/ml drug concentration, respectively.

These findings directly contradict those reported by Sjostrom et al., supra, for the formation of nanoparticles in presence of surfactants.

Example 10

Nanoparticle formation of a model Drug

30 mg isoreserpine (a model drug) is dissolved in 3.0 ml methylene chloride. The solution is added to 27.0 ml of human serum albumin solution (1 % w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000-18,000 psi while recycling the emulsion for at least 5 cycles. The number of cycles through the

homogenizer can vary from about one up to hundreds of cycles, depending on the size of the dispersion desired. The resulting system is transferred into a Rotary evaporator, and methylene chloride is rapidly removed at 40°C, at reduced pressure (30 mm Hg), for 20-30 minutes. The resulting dispersion is translucent, and the typical diameter of the resulting isoreserpine particles was 120-140 nm (Z-average, Malvern Zetasizer). The dispersion was filtered through a 0.22 micron filter (Millipore).

The sterile dispersion was further lyophilized for 48 hrs. without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

Example 11

Extremely Small particle formation with a model drug

The effect of ethanol addition on reducing particle size is demonstrated for Isoreserpine. Thus, 30 mg Isoreserpine is dissolved in 2.7 ml methylene chloride and 0.3 ml ethanol. The solution is added to 27.0 ml of human serum albumin solution (1 % w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification was performed at 9000-18,000 psi while recycling the emulsion for at least 5 cycles. The number of cycles through the homogenizer can vary from about one up to hundreds of cycles, depending on the size of the dispersion desired. The resulting system was transferred into a Rotary evaporator, and methylene chloride was rapidly removed at 40°C, at reduced pressure (30 mm Hg), for 20-30 minutes. The resulting dispersion was translucent, and the typical diameter of the resulting isoreserpine particles was 90-110 nm (Z-average, Malvern Zetasizer). The dispersion was filtered through a 0.22 micron filter (Millipore).

The sterile dispersion was further lyophilized for 48 hrs. without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

Example 12

Use of a Water miscible Solvent alone, supersaturated with drug - Not suitable for invention process

30 mg paclitaxel is dispersed in 0.6 ml ethanol. At this concentration (50 mg/ml), the paclitaxel is not completely soluble and forms a supersaturated dispersion. The dispersion is added to 29.4 ml of human serum albumin solution (1 % w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude dispersion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000-18,000 psi while recycling the emulsion for at least 6 cycles. The number of cycles through the homogenizer can vary from about one up to hundreds of cycles, depending on the size of the dispersion desired. The resulting system is transferred into a Rotary evaporator, and the ethanol is rapidly removed at 40°C, at reduced pressure (30 mm Hg), for 15-30 minutes. The resulting dispersion particle size is extremely broad, ranging from about 250 nm to several microns.

Observation under the microscope revealed the presence of large particles and typical needle shaped crystals of paclitaxel. These particles were too large for intravenous injection. This experiment demonstrates that the use of solvents such as ethanol that are freely miscible in water in the invention process results in the formation of large particles with very broad particle size distribution and as such cannot be used alone for the invention process. Thus, it is seen that the use of water miscible solvents is not preferred when used alone for the dissolution or dispersion of the drug component. The invention process requires that such solvents, when used, will preferably be mixed with essentially water immiscible solvents to allow efficient production of the invention nanoparticles.

Example 13

Use of a Water miscible Solvent alone containing dissolved drug - Not suitable for invention process

30 mg paclitaxel is dispersed in 1.3 ml ethanol. At this concentration (approx. 24.5 mg/ml), the paclitaxel is completely soluble in ethanol. The solution is added to 28.7 ml of human serum albumin solution (1 % w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude dispersion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000-18,000 psi while recycling the emulsion for at least 6 cycles. The number of cycles through the homogenizer can vary from about one up to hundreds of cycles, depending on the size of the dispersion desired. The resulting system is transferred into a Rotary evaporator, and the ethanol is rapidly removed at 40°C, at reduced pressure (30 mm Hg), for 15-30 minutes. The resulting dispersion particle size was extremely broad, ranging from about 250 nm to several microns. Observation under the microscope revealed the presence of large particles and typical needle shaped crystals of paclitaxel. These particles were too large for intravenous injection.

This example, in addition to Example 12 above, demonstrates that the use in the invention process of solvents such as ethanol that are freely miscible in water results in the formation of large particles with very broad particle size distribution and as such cannot be used alone for the invention process. Thus the invention process specifically excludes the use of water miscible solvents when used alone for the dissolution or dispersion of the drug component. The invention process requires that such solvents, when used, be mixed with essentially water immiscible solvents to enable formation of invention nanoparticles.

Example 14

Determination of Physical State of Paclitaxel

in Nanoparticle Form by X-Ray Powder Diffraction

Paclitaxel raw material is usually present as needle shaped crystals of varying sizes typically between 5-500 microns. The presence of crystals in a drug formulation for intravenous injection is obviously detrimental if crystals are present in size above a few microns due to potential blockage of capillaries. In addition, the solubility of drug crystals in general would be lower than for amorphous drug, thereby lowering the bioavailability of the drug following intravenous administration. It is also known that as the loading of the drug in a formulation is increased, the tendency for crystallization also increases. Thus it is advantageous that the formulation contain the drug in essentially amorphous form.

X-ray powder diffraction was used to determine the crystalline or non-crystalline nature of paclitaxel in the lyophilized powder formulation. The following samples were analyzed: Sample 1 - Paclitaxel powder; Sample 2 - Lyophilized serum albumin; Sample 3 - a physical mixture of paclitaxel and albumin; and Sample 4 - formulated paclitaxel. Each sample was x-rayed from 2° to 70° 2Q angles using CuKa radiation, an accelerating voltage of 40KeV/30mA, a step size of 0.05° 2Q and a data acquisition time of 2.0 seconds per step. Sample 1 showed strong peaks typical of a crystalline sample. The most intense paclitaxel peak was located at 5.1° 2Q. Sample 2 showed broad humps typical of amorphous material. Sample 3 showed largely the broad humps of Sample 2, but in addition, the peak at 5.1° 2Q of paclitaxel was visible. Sample 4, the formulated paclitaxel showed no evidence of crystallinity characteristic of paclitaxel and appeared identical to Sample 2, indicating the presence of substantially amorphous pharmacologically active agent in the formulated sample.

The amorphous nature of the nanoparticles produced according to the invention stands in direct contrast to the products produced by other methods described in the art for producing nanoparticles. For example, the use of grinding techniques, as described in U.S. Patent 5,145,684 (Liversidge et al.), and as described by Liversidge-Merisko et

al., *Pharmaceutical Research* 13(2):272-278 (1996), produces a substantially crystalline product.

Example 15

Treatment of Tumors in an Animal Model with Paclitaxel Nanoparticles

Nanoparticles of paclitaxel (the active ingredient of Taxol™) were prepared as described above in Example 1. This formulation of the drug was tested in a MX-1 human mammary tumor xenograft model in mice. The mice were implanted subcutaneously with the MX-1 mammary tumor and the treatment was initiated when the tumor reached approximately 150 - 300 mg in size. This occurred by day 12 and the treatment was initiated on day 13 after initial seeding.

Tumor bearing mice were treated with paclitaxel nanoparticles at a dose of 20 mg/kg, given by bolus intravenous injection as a suspension in saline for five consecutive days. The treated group included five animals. The control tumor bearing group of five animals received only saline on the same schedule. The size of the tumors was monitored as a function of time. The control group showed a tremendous increase in tumor weight. All the animals in this group were sacrificed between day 28 and day 39. The treatment group on the other hand showed remarkable efficacy as all animals had no measurable tumors by day 25. The animals in this group were all sacrificed on day 39, at which time they showed no evidence of recurrence and no evidence of tumor. The results are shown in Figure 1.

Example 16

Treatment of Rheumatoid Arthritis in an Animal Model with Paclitaxel Nanoparticles

Collagen induced arthritis model in the Louvain rat was used to test the therapeutic effect of paclitaxel nanoparticles on arthritis. The paw sizes of the experimental animals were monitored to evaluate the seriousness of arthritis.

After the arthritis was fully developed (usually ~9-10 days after collagen injection), the experimental animals were divided into different groups to receive either paclitaxel nanoparticles 1mg/kg q.o.d, or paclitaxel nanoparticles 0.5mg/kg + prednisone 0.2mg/kg q.o.d. (combination treatment) intraperitoneally for 6 doses, then one dose per week for three weeks. The paw sizes were measured at the beginning of treatment (day 0) and every time the drug was injected. One group received only normal saline as control. By the end of the experiment, the group receiving paclitaxel nanoparticles achieved a 42% reduction of paw size, the combination treatment group showed a 33% reduction of the paw size while the control group had about 20% increase of the paw size. Original paw size before arthritis was induced was 50%. The results are shown in Figure 2.

In conclusion, the paclitaxel-containing nanoparticles demonstrated therapeutic effect on arthritis. To avoid side effects of long term use of both paclitaxel and the steroid, it is probably better to choose a combination treatment to get similar effect but only half the dosage of each drug.

Example 17

In vivo Targeting of Nanoparticles

By incorporation of certain targeting moieties such as proteins, antibodies, enzymes, peptides, oligonucleotides, sugars, polysaccharides, and the like, into the protein coating of the nanoparticles, it is possible to target specific sites in the body. This targeting ability can be utilized for therapeutic or diagnostic purposes.

Example 18

Intravenous Delivery Systems Formulated From a Variety of Materials

The materials used for the preparation of intravenous delivery systems may be polymeric (e.g., polyethylene, polyvinyl, polypropylene tubing, and the like), or glass. Standard medical grade tubing is known to contain hydrophobic moieties on the inner surfaces thereof. These moieties are thus available to come in contact with the injection

solution. Indeed, such tubing is specifically tailored, as are the catheters, to present hydrophobic moieties in contact with the treatment solution so as to reduce the absorption of aqueous material to the tubing. However, any hydrophobic moieties in the treatment solution will likely bind to both the catheter tubing and other components of the delivery system. As a result, a substantial portion of a hydrophobic pharmacologically active agent can become sequestered in the inner walls of the tubing catheter and delivery vessel. Consequently, the dosing of hydrophobic pharmacologically active agents can be erratic, since a substantial portion of the active agent can become absorbed to the walls of the tubing. In critical therapeutic treatments, where the hydrophobic pharmacologically active agent is used to treat a disease, a significant reduction in the effective dose of active agent can lead to a therapeutic failure. The failure is particularly striking when employing therapeutic moieties which require that the active agent be present above a certain level, yet the therapeutic window is narrow.

A novel method for the intravenous introduction of a hydrophobic pharmacologically active agent has now been developed. By protecting the hydrophobic moieties of the active agent, through association with the hydrophobic moieties of a biocompatible coating (e.g., albumin), the propensity of the active agent to become attached to the tubing is dramatically reduced. Thus, the present invention enables the use of highly hydrophobic drugs, in combination with standard medical grade polymers and hydrophobic glasses, in which the drug is protected and therefore not absorbed onto the surface. The invention method comprises placing a protective coating of a biocompatible polymer (e.g., albumin) around the hydrophobic drug and placing the resulting composition in a hydrophobic polymeric delivery system. The invention methods are therefore capable of improving the delivery of a variety of hydrophobic therapeutics.

Example 19

Intravenous Administration of Therapeutics

Intravenous administration of therapeutics, for example, drugs, imaging agents, and the like, predisposes the therapeutic to at least one pass through the liver. As that therapeutic is filtered through the liver, a significant portion of that therapeutic is taken up and sequestered by the liver, and therefore, not available for systemic distribution. Moreover, once taken up by the liver, it is likely to be metabolized, and the resulting metabolic byproducts often have general systemic toxicities. By encapsulating the drug or other therapeutic agent in a coating according to the invention (e.g., using a protein such as albumin), liver sequestration upon intravenous administration is alleviated. Albumin, for example, is known to pass through the liver and become generally distributed throughout the patient. Thus, the sequestration of albumin by the liver does not occur to the same degree as toxic compounds or drugs which have hepatic receptors (or other mechanisms) which initiate processes which result in their removal from the blood stream. By protecting the therapeutic with a coating of a biocompatible polymer (e.g., a human albumin coating), the drug then bypasses the liver and is generally distributed through all organ systems. In accordance with one aspect of the present invention, there is provided a novel method for bypassing the liver, which comprises encapsulating a drug in a human liver albumin (essentially a physiological component). In this way, more of the drug becomes available for systemic therapy. In addition to the increased availability of the drug, there is a decrease in the production of metabolic byproducts of hepatocellular drug degradation. Both the increase in liver bypass and decrease in byproducts of drug metabolism provide a synergistic improvement in the overall drug efficacy. This improved efficacy extends to all drugs and materials that are encapsulated in human albumin.

Example 20

Reducing Myelosuppressive Effects
and General Toxicity of Drugs

Several chemotherapeutic drugs have dose limiting toxicity due to their myelosuppressive effects. Paclitaxel (the active ingredient of Taxol™) is a classic example of such a drug. When administered in its currently approved formulation of cremaphor/ethanol (i.e., Taxol™), paclitaxel produces myelosuppressive effects that limit the repeat administration of the drug and preclude retreatment of a patient for at least 3 weeks in order to allow blood counts of the patient to return to normal. It was postulated that due to the non-toxic compatible nature of the drug carrier of the present invention, viz. human albumin, the toxic side effect of myelosuppression may be greatly reduced.

Sprague dawley rats were given paclitaxel in commercial formulation (available from Bristol Myers Squibb (BMS) in cremaphor/ethanol) or prepared by the invention method as nanoparticles with albumin. Both formulations were administered by tail vein injection. A single dose level of 5 mg/kg was administered for the BMS formulation, whereas two dose levels of 5 mg/kg and 12 mg/kg were administered for the invention formulation (Capxol). The white blood cell counts of the rats were monitored daily after administration as an index of myelosuppression.

For the BMS formulation (5 mg/kg) it was found that the WBC counts dropped by 47.6% and 63.5% on day 1 and day 2 after administration, respectively, whereas for the Capxol formulation at 5 mg/kg, the WBC counts increased by 14.7% and 2.4% on day 1 and day 2, respectively. For the higher dose Capxol at 12 mg/kg, the WBC counts increased by 6.5% and 3.6% on day 1 and day 2, respectively.

These results indicate that short term myelosuppression is greatly reduced by administering the drug in the present invention formulation.

Another indicator of general toxicity is the body weight of the animal. Body weights of the rats were also monitored following administration of paclitaxel. At a dose of 5 mg/kg, the BMS formulation resulted in a reduction of body weight by 10.4% in 3 days following administration, whereas the same dose of paclitaxel administered in the invention formulation (Capxol) resulted in only a 3.9% drop in body weight, indicating the greatly reduced toxicity of the invention formulation.

Example 21

Administration of Bolus dose of Nanoparticle Formulation

The anticancer drug, paclitaxel, in its commercial BMS formulation with Cremaphor/ethanol, cannot be administered as an intravenous bolus. This is due to the extensive toxicity of the vehicle which results in severe anaphylactic reactions and requires patients receiving the drug to be pre-medicated with steroids, antihistamines, and the like. The BMS formulation is administered as an intravenous infusion lasting anywhere from 1 hour to 24 hours. In contrast, formulations according to the present invention, due to the use of a non-toxic carrier, can be administered to a patient readily as an intravenous bolus (i.e., in a period less than 1 hour) without the toxicity problems seen in the BMS formulation that is used clinically today.

The effective dose of paclitaxel for a patient typically lies between 200-500 mg, depending on the patient body weight or body surface. The BMS formulation has to be administered at a final dosing concentration of 0.6 mg/ml, requiring large infusion volumes (typically in the range of about 300-1000 ml). In contrast, invention formulations (e.g., Capxol) do not have these limitations and can be administered at a desired concentration. This enables clinicians to treat patients by a rapid intravenous bolus that can be administered in as little as a few minutes. For example, if the invention formulation is reconstituted to a dosing concentration of 20 mg/ml, the infusion volume for a total dose of 200-500 mg is only 10-25 ml, respectively. This is a great advantage in clinical practice.

Example 22

Reduction in Toxicity of Paclitaxel in the Nanoparticle Formulation Compared to the Commercial Cremaphor/Ethanol Formulation

It is well known that the anticancer drug, paclitaxel, in its commercial BMS formulation with Cremaphor/ethanol, has extensive toxicity which results in severe anaphylactic reactions and requires patients receiving the drug to be pre-medicated with steroids, antihistamines, and the like. The toxicity of the BMS formulation was compared to the nanoparticle formulation of the present invention.

Thus, the formulations were injected intravenously through the tail vein of C57BL mice at different dose levels and toxic effects were monitored by general observation of mice after the injection.

For the BMS formulation, a dose of 30 mg/kg was uniformly lethal within 5 minutes of intravenous administration. For the same dose, the nanoparticle formulation according to the invention showed no apparent toxic effects. The nanoparticle formulation at a dose of 103 mg/kg showed some reduction in body weight of the mice, but even this high dose was not lethal. Doses of approximately 1000 mg/kg, 800 mg/kg and 550 mg/kg were all lethal but differing in time to lethality, which ranged between a few hours to 24 hours. The lethal dose of the invention formulation is greater than 103 mg/kg but less than 550 mg/kg.

Thus, the lethal dose of the invention formulation of paclitaxel is substantially higher than that of the commercial BMS formulation. This has great significance in clinical practice where higher doses of chemotherapeutic drugs may be administered for more effective oncolytic activity with greatly reduced toxicity.

Example 23

Preparation of Nanoparticles of Cyclosporine

(Capsorine I.V.) by High Pressure Homogenization

30 mg cyclosporine is dissolved in 3.0 ml methylene chloride. The solution is then added into 27.0 ml of human serum albumin solution (1% w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer model: Tempest I.Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification was performed at 9000-18,000 psi while recycling the emulsion for at least 5 cycles. The number of cycles through the homogenizer can vary from about one up to hundreds of cycles, depending on the size of the dispersion desired. The resulting system was transferred into a Rotavap and methylene chloride was rapidly removed at 40°C, at reduced pressure (30 mm Hg), for 20-30 minutes. The resulting dispersion was translucent and the typical diameter of the resulting cyclosporine particles was 160-220 (Z-average, Malvern Zetasizer).

The dispersion was further lyophilized for 48 hours, without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

Example 24

Preparation of Nanodroplets of Cyclosporine

(Capsorine Oral) by High Pressure Homogenization

30 mg cyclosporine is dissolved in 3.0 ml of a suitable oil (sesame oil containing 10% orange oil). The solution is then added into 27.0 ml of human serum albumin solution (1% v/w). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I.Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000-18,000 psi while recycling the emulsion for at least 5 cycles. The number of cycles through the homogenizer can vary from about one up to hundreds of cycles,

depending on the size of the dispersion desired. The resulting dispersion had a typical diameter of 160-220 (Z-average, Malvern Zetasizer).

The dispersion could be used directly or lyophilized for 48 hours by optionally adding a suitable cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline.

Example 25

Pharmacokinetic (PK) Data for Cyclosporine Nanoparticles (Capsorine I.V.) Following Intravenous Administration Comparison with Sandimmune I.V. (Currently Marketed Formulation by Sandoz)

Nanoparticles of cyclosporine (Capsorine I.V.) prepared as described above (Examples 22 and 23) were reconstituted 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	2,853
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.).

Example 26

Pharmacokinetic (PK) Data for Cyclosporine Nanodroplets (Capsorine Oral) Following Oral Administration Comparison with Neoral (Currently Marketed Formulation 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 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 Oral	3,195	887
Neoral	3,213	690

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

Example 27

Intravenous Administration of Invention Paclitaxel Formulation (Capxol™) in Human Subjects

In a Phase I clinical study, an invention formulation of paclitaxel (Capxol™) was administered by intravenous injection to 17 human subjects exhibiting advanced metastasis (Of the 17 subjects enrolled in the Phase I clinical study, 6 subjects had malignant melanoma and the remaining 11 subjects had metastatic breast cancer). The initial dose level (paclitaxel single dose) administered to each subject was 135 mg/m^2, and the subsequent doses were escalated to the next higher paclitaxel single

dose level (per conventional paclitaxel dose regimen) if there were no significant adverse effects in the subject.

The maximum single dose administered was 375 mg/m^2 paclitaxel, with this dose being given in saline over a period of 30 minutes or less. This maximum single dose is significantly higher than the approved dose of paclitaxel (175 mg/m^2) administered as part of the conventional formulation, Taxol™.

No significant adverse events were noted in these subjects at this 375 mg/m^2 dose level, except for a small number of patients who exhibited some neurotoxicity (neuropathy). Accordingly, administration in invention formulations of paclitaxel at single dose levels which are as high as 500 mg/m^2, 1000 mg/m^2, 1500 mg/m^2, or 2000 mg/m^2 or higher, and which are less than the level at which 50% or more of the patients receiving this formulation exhibit a significant adverse event (e.g., a dose limiting toxicity), are contemplated.

One of the dose limiting toxicities in the administration of paclitaxel as part of the conventional formulation, Taxol™, is the neurotoxicity (neuropathy) of Taxol™, which is seen in up to 70% of patients receiving Taxol™. Surprisingly, an invention formulation of paclitaxel (Capxol™) shows significantly reduced incidence of neurotoxicity (neuropathy) even when utilizing higher paclitaxel dose levels than are permitted with Taxol™.

Another significant dose limiting toxicity for Taxol™ is myelosuppression, which is seen in up to 90% of patients receiving Taxol™ at a dose of 175 mg/m^2 (i.e., subject's neutrophil count drops to < 2000/mm^3). As evidenced by white blood cell (WBC) and platelet counts, invention formulations of paclitaxel (Capxol™) are surprisingly found to be significantly less myelotoxic than Taxol™, even for patients receiving a much higher paclitaxel dose in accordance with the present invention, i.e., up to 375 mg/m^2.

Two of the malignant melanoma subjects being treated with the invention formulations of paclitaxel (Capxol™) did not exhibit any progression of the malignant

melanoma for periods ranging from more than 15 weeks to more than 30 weeks. It is surprising that administration of the invention formulations of paclitaxel (Capxol™) generates a response in these patients because paclitaxel, administered in the conventional formulation of Taxol™, previously had not been found to generate a significant response in the treatment of malignant melanoma.

At least 2 of the 11 metastatic breast cancer subjects had a definitive response with administration of the invention formulations of paclitaxel (Capxol™). Two of the 11 metastatic breast cancer subjects were taken off the study due to progression of the disease, and the rest maintained stable disease at the time of evaluation. Thus, the invention formulations of paclitaxel (Capxol™) showed definite benefit to these cancer subjects.

Pharmacokinetic data in these patients showed an increase in the area under the curve (AUC) (e.g., amount of drug retained in patient's blood over time) with increasing dose. Therapeutic levels of the drug (>50 ng/ml) were maintained in whole blood up to 48 hours after dosing.

In one patient with breast cancer who had severe psoriasis over the trunk and limbs, it was surprisingly noted that her psoriasis was significantly reduced after administration of the first course of treatment of the invention formulation (Capxol™). The psoriasis remained in control over the next several courses of treatment of the invention formulation (Capxol™), indicating that paclitaxel (administered as Capxol™) can have a positive effect in the treatment of psoriasis. Thus, paclitaxel (administered as Capxol™) can be useful in the treatment of psoriasis and other proliferative diseases (e.g., multiple sclerosis, vascular restinosis, and the like).

Example 28

Intraarterial Administration of Invention Paclitaxel Formulation (Capxol™) in Human Subjects

In a further Phase I clinical study, an invention formulation of paclitaxel (Capxol™) was administered intra-arterially to more than 25 patients exhibiting primary and/or secondary liver tumors and/or solid tumors with local-regional involvement. The clinical study protocol called for administration of the invention formulation of paclitaxel (Capxol™) by intra-arterial injection (chemo-embolization) into the artery feeding the tumor that was to be treated. For example in patients with hepatic disease, the hepatic artery could be catheterized and the drug injected directly into the artery feeding the tumor.

Starting with a dose of 90 mg of paclitaxel per patient in the initial course of treatment, the dose was escalated for subsequent courses of treatment to over 300 mg per patient. Tumor response to this course of treatment in these patients was assessed by X-ray, MRI, CT scan and/or angiography.

The treated patients included patients with hepatocellular carcinoma, multifocal hepatoma, metastatic breast cancer, prostatic cancer, pancreatic cancer, non-small cell lung cancer (squamous cell cancer), colon cancer, renal cancer, intestinal sarcoma, esophageal cancer, melanoma, ependymoma, and head & neck cancer. Surprisingly, responses to treatment (e.g., reductions in cancer size, and the like) were observed for most of these patients, including patients with lung cancer, prostatic cancer, breast cancer, and/or hepatic disease. In general, intra-arterial injection was remarkably well tolerated by the treated patients, as toxicity in these patients was very limited. Late toxicity included slight alopecia, a slight reduction in WBC and a sense of fatigue lasting 4-5 days.

It is to be noted that intra-arterial injection of the invention formulation (Capxol) of paclitaxel allowed the administration of the drug substance paclitaxel in a manner not previously possible. Previously only a very limited dose of paclitaxel could be administered intra-arterially due to the low solubility of paclitaxel. The

invention formulation allows intra-arterial administration at much higher doses thereby allowing a more efficacious treatment of the disease. Furthermore, this mode of treatment results in responses to cancers e.g., prostatic and pancreatic cancer which are normally considered nonresponsive.

Example 29

Treatment of Diseases with Invention Paclitaxel Formulation (Capxol™) Where Use of Steroids is Contraindicated

Little progress has been made in the development of treatment regimens which are effective to reduce and/or inhibit progression of metastatic melanoma following conventional chemotherapy in patients who have this disease. Interferon and interleukin have been utilized alone or in combination for such treatment. Unfortunately, the response rate (i.e., the percentage of patients exhibiting a positive treatment response (e.g., reduction and/or inhibition of the progression of metastatic melanoma following conventional chemotherapy) after administration of the drug) is less than 25%. Similarly, paclitaxel (administered in its currently approved formulation, Taxol™) has been evaluated for efficacy against metastatic melanoma, and has been shown to have definite but modest activity with a response rate of 15%. Further, the response rate utilizing interleukin-2 (IL-2) is similar, but, unlike complete responses (CRs) induced by single cytotoxic agents, CRs induced by IL-2, although uncommon, can be durable.

The combination of paclitaxel (administered in its conventional, approved formulation, Taxol™) and IL-2 has not previously been evaluated for treatment of metastatic melanoma because of the requirement for corticosteroid premedication prior to administration of Taxol™. Corticosteroid premedication is required to prevent anaphylactic-like hypersensitivity reactions associated with the cremophor vehicle of Taxol™. The corticosteroids introduced due to this requirement cause lysis of LAK cells, resulting in the loss of any benefit from administration of IL-2. Thus, Taxol™ cannot be used in combination with drugs like IL-2 or interferon.

The invention formulations of paclitaxel (e.g., Capxol™) are cremophor-free formulations of paclitaxel which are freely dispersible in aqueous media. Because the invention formulations are cremophor-free, the risk of hypersensitivity reactions on administration of the invention formulations is remote. Thus, corticosteroid premedication is not required with administration of invention formulations of paclitaxel (e.g., Capxol™). This discovery permits treatment of patients in need thereof with a combination of paclitaxel (Capxol™) and IL-2 (e.g., metastatic melanoma patients who have failed conventional chemotherapy, patients with renal cell carcinoma, and the like).

For example, metastatic melanoma patients who have failed conventional chemotherapy can be treated with a combination of paclitaxel (Capxol™) and IL-2. Dosing of these patients can be accomplished by the following regimen. An invention formulation of paclitaxel (e.g., Capxol™) is administered intravenously over approximately 30 minutes at an initial dose of 200 mg/m^2 on day 1 (before start of IL-2 dosing) and at a dose of 100 mg/m^2 on day 6 (upon completion of IL-2 dosing). IL-2 is administered intravenously at 18 MIU/m^2 over 6 hours beginning after administration of the initial dose of paclitaxel, followed by 18 MIU/m^2 administered intravenously over 12 hours, followed by 18 MIU/m^2 administered intravenously over 24 hours, followed by 4.5 MIU/m^2/day administered intravenously over 3 consecutive days, with the total administration time of IL-2 being 120 hours from day 1 to day 6. Other suitable schedules for drug administration reported in the literature can also be utilized.

As a further example, patients with renal cell carcinoma can be treated with a combination of paclitaxel (e.g., Capxol™) and IL-2. Conventionally, IL-2 is used for the treatment of these patients. However, paclitaxel (administered in its approved formulation, Taxol™) cannot be administered to these patients, alone or in combination with IL-2, because of the need for corticosteroid premedication prior to administration of Taxol™, as discussed above. But, as discussed above, invention formulations of paclitaxel (e.g., Capxol™) do not require corticosteroid

premedication, and therefore are suitable for administration in combination chemotherapy with IL-2 for the treatment of these patients.

Generalizing the foregoing exemplary combination therapeutic treatments of specific diseases, any situation requiring treatment of patients utilizing IL-2, interferon or other cytokine biochemotherapy agents is also suitable for the treatment of these patients utilizing invention formulations of taxanes (e.g., paclitaxel, docetaxel, and like taxanes which require steroid premedication) in combination therapy with the cytokine biochemotherapy. Further, in any situation where treatment of a disease with taxanes (e.g., paclitaxel (in its approved formulation Taxol™), docetaxel (in its approved formulation Taxotere™), and other taxanes which require steroid premedication) is desirable in conjunction with biochemotherapy agents (e.g., interleukins, interferons, other cytokines, and the like), use of invention formulation of taxanes obviates the need for steroid administration and makes possible the treatment of patients with combination chemotherapy. This combination chemotherapy can provide added benefits to the treated patient.

This example demonstrates how the use of invention formulations is possible in any situation where steroid medication is contraindicated.

Example 30

Administration of Taxanes (e.g., Paclitaxel) Without Premedication or GSF-Support

Hypersensitivity reactions are a problem associated with administration of paclitaxel (in its commercially available formulation, Taxol™), docetaxel (in its commercially available formulation, Taxotere™), and other taxanes.

Taxol™, the commercially available formulation of the anti-cancer agent paclitaxel, is available as a drug which can be dissolved prior to administration utilizing cremophor (polyethoxylated castor oil) as a surfactant and ethanol as a vehicle. Hypersensitivity reactions (e.g., due to drug- and/or vehicle-induced toxicity) to Taxol™ occur in about 40% of all patients receiving Taxol™. Because of this documented hypersensitivity reaction, all patients to whom Taxol™ is to be

administered are required to take premedication that comprises corticosteroids and antihistamines prior to receiving Taxol™.

Similarly, Taxotere™, the commercially available formulation of the anti-cancer agent docetaxel, is another taxane that is dissolved in the surfactant Tween-80. Because of analogous hypersensitivity reactions, administration of Taxotere™ also requires steroid premedication.

Surprisingly, the invention formulations of taxanes (e.g., Capxol™ (which comprises nanoparticles of paclitaxel stabilized by human serum albumin), and the like) were found surprisingly to not induce any hypersensitivity reactions in human patients. As a result, patients receiving the invention formulations of taxanes (e.g., paclitaxel (in Capxol™ formulation), docetaxel, and the like) need not be premedicated with steroids.

Like hypersensitivity reactions, myelosuppression is a problem (i.e., a dose limiting toxicity) associated with administration of paclitaxel in its commercially available formulation, Taxol™.

A large number of patients receiving Taxol™ are required to take G-CSF support to prevent excessive myelosuppression. For example, in those patients receiving Taxol™ without G-CSF support, the neutrophil counts or absolute granulocyte counts may drop to dangerously low levels (i.e., less than about 500/mm^3).

Thus, it was surprisingly found that the degree of myelosuppression in patients receiving the invention formulation of paclitaxel (e.g., Capxol™) is much lower than for patients receiving the same dose of paclitaxel in the commercially available formulation of paclitaxel, Taxol™. Unlike patients receiving Taxol™, patients receiving invention formulations of paclitaxel (e.g., Capxol™), even at doses as high as 375 mg/m^2, exhibit little myelosuppression, and require no G-CSF support. Accordingly, it can be surprisingly noted that patients receiving invention formulations of taxanes (e.g., paclitaxel, docetaxel, and the like) do not require

cytokine (e.g., G-CSF, and the like) support or any other means of support required to avoid excessive myelosuppression.

Example 31

Localized Treatment of Brain Tumors and Tumors Within The Peritoneum

Delivering pharmacologically active agents (e.g., antiinfectives, immunosuppressives, chemotherapeutic agents, and the like) locally to a specific treatment site (e.g., localized tumor site, and the like) is an effective method for long term exposure to the drug while minimizing dose limiting side effects. The biocompatible materials discussed above can be employed in several physical forms (e.g., gels (crosslinked or uncrosslinked), and the like) for the purpose of facilitating this controlled local delivery. When so utilized, these biocompatible materials provide matrices from which pharmacologically active agent(s) (e.g., taxanes (paclitaxel, docetaxel, and the like), and the like) can be locally released and/or dispersed (e.g., at the site of the solid tumor (e.g., brain (brain cancer), peritoneum (ovarian cancer), and the like), and the like) by diffusion across and/or degradation of the matrix.

Thus, the invention formulations of paclitaxel (e.g., Capxol™) can be dispersed within a matrix of the biocompatible material(s) discussed above. This matrix provides a sustained release formulation of paclitaxel for the treatment of brain tumors and tumors within the peritoneal cavity (e.g., ovarian cancer, metastatic diseases, and the like). When temperature sensitive materials are utilized in the formation of this matrix, the invention formulations of paclitaxel (e.g., Capxol™) can be injected in a liquid formulation of the temperature sensitive materials which gels at the tumor site and provides for slow release of Capxol™.

Capxol™ (an invention formulation of paclitaxel) was reconstituted in a 1% solution of sodium alginate. The solution was then pumped through a device to make gel beads of the Capxol™/alginate solution in aqueous calcium chloride.

Some beads containing Capxol™ were separated from the solution and placed in culture media. The release of paclitaxel from the beads containing Capxol™ into the

media was measured over time at 37°C. It was found that sustained release of paclitaxel could be obtained from the beads containing Capxol™ for periods of up to 28 days.

Additional beads containing Capxol™ were separated from the solution and implanted into the peritoneal cavity of rats to determine release of paclitaxel from within the beads. The rats were sacrificed 2 weeks later and the gelled beads retrieved. Measurement of paclitaxel within the beads showed that most of the drug had been released into the peritoneal cavity of the rats.

Example 32

Reduction of Dosing Cycle Time and Increase in Dose Levels in Administration of Taxanes

Due to the low toxicity of invention formulations of paclitaxel (or other pharmacologically active agents, e.g., docetaxel (commercially available as Taxotere™), and the like), it is possible to reduce the associated cycle time (i.e., the time between consecutive administrations of doses of the pharmacologically active agent) and/or increase the associated dose levels (i.e., the amount of each dose of pharmacologically active agent).

In the case of paclitaxel administered in its approved formulation, Taxol™, or docetaxel administered in its approved formulation, Taxotere™, the typical cycle time is 3 weeks. However, invention formulations of the taxane (e.g., paclitaxel, docetaxel, and the like) can be administered with cycle times in the range of from about 1 day to about 3 weeks or more, with a preferable cycle time of about 3 to about 15 days, as required to provide maximum benefit from treatment to the patient in need thereof. For example, doses of 25-250 mg/m^2 of paclitaxel in invention formulations can be administered every 1-20 days in patients suffering from any disease in which treatment with paclitaxel shows some benefit.

In the case of paclitaxel administered in its approved formulation, Taxol™, the typical dose level is 100-135 mg/m^2 with a cycle time of 1 week. However, invention formulations of the taxane (e.g., paclitaxel, docetaxel, and the like) can be

administered with this same cycle time in doses which exceed typical dose levels (e.g., doses greater than 135 mg/m^2 and up to 400 mg/m^2 or greater, as required to treat patients suffering from any disease in which treatment with a taxane shows some benefit.

Example 33

Oral Administration of Taxanes

Oral administration of taxanes (e.g., paclitaxel, docetaxel, and the like) has historically been challenging.

However, it has been discovered that invention formulations of a taxane (e.g., paclitaxel, docetaxel, and the like) can be administered orally in combination with oral cyclosporin and/or oral FK506 (i.e., tacrolimus).

Invention formulation(s) of the taxane and/or cyclosporin and/or FK506 can be prepared in accordance with the invention methods. These formulation(s) can be prepared as separate formulations (for oral administration in series), or as a combination formulation comprising the taxane and at least one of cyclosporin and FK506 (for oral administration in parallel).

Once these formulation(s) have been prepared, they can be administered to a patient suffering from any disease in which treatment with a taxane shows some benefit, utilizing various dose amounts and cycle times necessary to effect treatment of the disease.

Without limiting the scope of this example, the oral administration of the taxane formulation is believed to be facilitated by the oral administration (either in parallel (i.e., concurrent with administration of the taxane formulation) or in series (i.e., prior to administration of the taxane formulation)) of cyclosporin and/or FK506. Cyclosporin and/or FK506 are believed to inhibit the function of P-glycoprotein efflux pumps on intestinal cells. Uninhibited, these P-glycoprotein efflux pumps

would likely pump any absorbed paclitaxel formulation out of its associated intestinal cell.

Example 34

Reconstitution and Use:

An exemplary invention composition, referred to herein as ABI-007, is prepared in dosage forms containing 30 mg, 100mg or 300mg of paclitaxel in a vial.

When reconstituted with 0.9% Sodium Chloride Injection, USP, ABI-007 forms a colloidal suspension of paclitaxel stabilized with Human Albumin, USP. The formulation contains no other added excipients. The sterility of the product is assured by an aseptic manufacturing process and sterile filtration.

The two major components of ABI-007 are paclitaxel and Human Albumin, USP. Since human albumin is soluble in Sodium Chloride Injection, USP, ABI-007 can be reconstituted to the desired concentration of paclitaxel, which is limited only by the solubility limits for human albumin.

Administration of ABI-007 to the patient can be accomplished by intravenous infusion at a dosage directed in the clinical protocol. At any given dose of paclitaxel in mg/m^2, the total mg of paclitaxel to be administered should be calculated by the physician, using the height/weight conversion chart for body surface area of the patient.

Reconstitution and use of invention formulation can be accomplished as follows:

Calculate the patient's body surface area by using the height/weight chart;

Calculate the total mg required by the patient;

Calculate the total number of vials required by the patient;

Under a biological safety cabinet, using sterile technique, reconstitute each vial by injecting a suitable quantity of 0.9% Sodium Chloride Injection, USP to achieve the desired administration concentration (0.2 – 40 mg/ml) with a preferred range of 2-10 mg/ml;

After reconstitution, agitate by gentle mixing before use (avoid generation of foam);

Allow 20 minutes for complete suspension and dissolution;

Gently agitate again to ensure complete resuspension prior to administration;

The reconstituted sample should be milky and homogeneous without visible particulates. If particulates are visible, the vial should be mildly agitated again to ensure complete re-suspension, prior to use;

Inject the reconstituted ABI-007 into an empty sterile, plastic type IV bag, using an injection site. Inject perpendicularly into the center of the injection site, to avoid dislodging plastic material into the IV bag. Use a new 60 cc syringe after every two injections into the I.V. bag;

Remove the injection site; and

Administer the reconstituted ABI-007 solution by IV infusion over a desired period (e.g., bolus injection, 30 min, 1 hr, 2hr, 3 hr, 6hr, 24hr, 48 hr, 72hr or 96 hour infusions). Preferred administration period is no greater than about 3 hours. Do not use in-line filters, as they are not necessary.

Example 35

Stability of Invention compositions

Invention compositions and dosage forms in polymeric or glass containers were diluted with sterile normal saline to concentrations of 0.5, 1, 5, 10, and 15 mg/ml.and stored at room temperature and under refrigerated conditions. The compositions were

found to be homogeneous and stable for at least 24 hours to three days under these conditions. Particle size measurements performed at several time points indicated no change in size distribution. No precipitation was seen under these conditions.

In addition, the diluted compositions were stable in the presence of different polymeric materials such as teflon, silastic, polyethylene, tygon, and other standard infusion tubing materials. The compositions were stable under frozen, refrigerated as well as room temperature conditions.

Stability studies of the non-diluted invention compositons show stability under refrigerated and room temperature conditions for over 1 year. Freezing, refrigeration or room temperature storage does not adversely affect the product.

Example 36

Dissolution and Release of Drug from Invention Compositions

Once the compositions are diluted with saline or dextrose or other aqueous medium, the drug becomes available or is released in solution. Different dissolution media may be utilized for example, media containing surfactants such as tweens, proteins such as serum albumin, etc. to measure release and availability of the drug. Dissolution and release testing are performed by techniques well described in the art.

It is found that a majority of the drug is released from invention compositions of paclitaxel within 24 hours.

Example 37

Dosage forms

Dosage forms of invention compositions are prepared in unit vessels of glass, metallic, organic, inorganic or polymeric origin or combinations thereof with suitable closures that are glass, metallic, organic, inorganic or polymeric in origin or

combinations thereof. Depending on the potency of invention composition drugs, the unit vessels may contain between 1 mg to 1000 mg of active drug. Suitable dosage forms include but are not limited to 5mg, 10mg, 20mg, 30mg, 50mg, 80mg, 100mg, 150mg, 200mg, 250mg, 300mg, 350mg, 400mg, 450mg, 500mg, 600mg, 800mg, 1000mg. These dosage forms are stable when stored frozen, refrigerated or at room temperature and for select periods at elevated temperatures.

Example 38

Anti-tumor efficacy of ABI-007 in Animals

The pharmacology of paclitaxel is well understood and is described in the literature. Paclitaxel is a novel anti-microtubule agent that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. The stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for the vital interphase and mitotic cellular function. In addition, paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple arrays of microtubules during mitosis. It has recently been reported that paclitaxel facilitates apoptosis in cells containing the Bcl-2 gene. It is postulated that paclitaxel phosphorylates the Bcl-2 gene and induces programmed cell death. Several tumors are known to be Bcl-2 positive (prostate tumors).

A pilot study has been conducted to determine the efficacy of the ABI-007 formulation of paclitaxel. Female athymic NCr-nu mice were implanted with MX-1 human mammary tumor fragments and treated with daily (five days) injections of ABI-007, ABI-007 vehicle and TAXOL. The efficacy of ABI-007 was demonstrated with 100% survival of the animals in the high dose group (45 mg/kg/day) of both formulations of ABI-007. In contrast, no animals survived after receiving vehicle, and only 40% of animals survived on the highest dose of TAXOL (30 mg/kg/day).

The antitumor activity of ABI-007 was also demonstrated in an *in vitro* study. Murine leukemia cells (L1210) were cultured in suspension and dilutions of

ABI-007 or TAXOL (0.01 to 0.5 μg/mL) were added to the cell cultures in Falcon 96-well plates. Cells were counted after 48 hours.

Both ABI-007 and TAXOL exhibited antitumor activity in L1210 murine leukemia cells. The IC_{50} for ABI-007 and TAXOL were 0.014 μg/mL and 0.010 μg/mL respectively. The slightly lower IC_{50} for TAXOL is probably caused by the toxic effect of the Cremophor/ethanol vehicle.

Example 39

Pharmacokinetics and Product Metabolism in Animals

Two studies were performed to compare the pharmacokinetics and tissue distribution of ^{3}H-paclitaxel formulated in ABI-007 and TAXOL. Male rats received intravenous injections of either 10 mg/kg or 5 mg/kg of ^{3}H-TAXOL. A third group of rats received intravenous injections of 5 mg/kg ^{3}H-ABI-007.

Levels of both total radioactivity and paclitaxel declined bi-phasically in blood of rats following 5 mg/kg IV bolus doses of either ^{3}H-TAXOL or ^{3}H-ABI-007. However, the levels of both total and paclitaxel in blood were significantly lower for ^{3}H-ABI-007 at a similar dose as ^{3}H-TAXOL dose.

^{3}H-ABI-007 and ^{3}H-TAXOL exhibited a similar pattern of paclitaxel metabolism into highly polar metabolite(s) as measured by blood HPLC profile; however, metabolism appeared significantly slower for ^{3}H-ABI-007 with 44.2% of blood radioactivity remaining as paclitaxel at 24 hours post-dose versus 22.4% for ^{3}H-TAXOL. The excretion of radioactivity occurred only minimally in the urine and predominantly in the feces for ^{3}H-ABI-007, which is similar to the reported excretion patterns for ^{3}H- TAXOL.

The blood kinetics for total radioactivity and paclitaxel following IV administration of ^{3}H-ABI-007 or ^{3}H- TAXOL at 5 mg/kg are presented in Table 3.

Table 3. Kinetics for Total Radioactivity and Paclitaxel Following Intravenous Dosing

Treatment	AUC_{0-24} (μg eq.hr/mL)	Extrapolated C_0 (μg eq/mL)	Observed C_{max} (μg eq/mL)	Observed T_{max} (hr)	$t_{1/2}\beta$ (hr)
Total Radioactivity					
^{3}H-ABI-007	6.1	7.6	4.2	0.03	19.0
^{3}H-TAXOL	10.2	19.7	13.5	0.03	19.7
Paclitaxel					
^{3}H-ABI-007	3.7	7.0	4.0	0.03	11.4
^{3}H-TAXOL	5.4	17.1	11.8	0.03	7.2

Tissue radioactivity levels are higher following ^{3}H-ABI-007 administration than ^{3}H-TAXOL administration for 9 of 14 tissues. ^{3}H-ABI-007 was present in significantly greater quantities than ^{3}H-TAXOL in prostate, spleen, and pancreas and to a lesser extent in bone, kidney, lung, heart and muscle. The tissue/blood concentration ratios were higher in all tissues for the ^{3}H-ABI-007 dosed animals as a result of the lower blood levels. This supports the rapid distribution of ^{3}H-ABI-007 from the blood to the tissues suggested by the blood kinetic data. ^{3}H-paclitaxel formulated in ABI-007 showed a similar pharmacokinetic profile to ^{3}H- paclitaxel formulated in TAXOL, but tissue/blood concentration ratios and metabolism rates differ significantly. A significantly lower level of total radioactivity was detected for ABI-007-treated animals than for TAXOL-treated animals in the 2-minute post administration blood sample, indicating that the ^{3}H-ABI-007 is more rapidly distributed out of the blood.

In a third study, four additional groups of rats were treated with a single bolus dose of 9.1 mg/kg, 26.4 mg/kg, 116.7 mg/kg, and 148.1 mg/kg of paclitaxel in ^{3}H-ABI-007. Blood was collected from the tail vein and the AUC_{0-24} was calculated. At 24 hours, blood samples were collected, extracted, and the extract analyzed by HPLC to determine the level of parent compound in the blood. Higher bolus doses of ^{3}H-TAXOL were not tested because of the death of 5 of 14 animals at 10 mg/kg in a previous study.

The blood kinetics for total radioactivity and paclitaxel following IV administration of ^{3}H-ABI-007 are presented in Table 4.

Table 4. Kinetics for Total Radioactivity and Paclitaxel Following IV Dosing

Group/Dose (mg/kg)	AUC_{0-24} (μg eq.hr/mL)	Extrapolated C_0 (μg eq/mL)	Observed C_{max} (μg eq/mL)	Observed T_{max} (hr)	$t_{1/2}\beta$ (hr)
A/9.1	11.5	10.2	7.19	0.03	22.3
B/26.4	43.5	44.8	29.5	0.03	16.0
C/116.7	248.9	644.6	283.3	0.03	8.48
D/148.1	355.3	1009.8	414.2	0.03	9.34

As the dose of paclitaxel was increased, the area under the curve was proportionally increased. The level of parent compound after 24 hours was increased by a factor of 8.5 (0.04 ppm - 0.34 ppm), going from the 9 mg/kg dose to the 148 mg/kg dose.

^{3}H-ABI-007 was well-tolerated as a single IV bolus at all four doses tested. The plot of the AUC for total radioactivity versus IV dose administered shows a linear, but disproportionate response with a slope greater than one. This response was also observed when comparing 5 and 10 mg/kg doses of ^{3}H-TAXOL tested in previous studies. HPLC analysis of 24-hour post-dose blood samples showed a significant percentage (7-19%) of blood activity as unchanged paclitaxel, indicating that paclitaxel in ABI-007 is slowly metabolized in vivo and that total exposure to paclitaxel is significant for ABI-007-treated animals. Paclitaxel is not a new drug substance. Preclinical studies of ABI-007 have therefore been limited to specific studies which evaluate the toxicity of the new formulation and the potential use of higher doses of paclitaxel in humans than is currently approved.

Example 40

Toxicology in Mice

A pilot study was conducted to compare the LD_{50} of ABI-007, TAXOL and their carrier vehicles following a single bolus intravenous administration into mice. The results indicated that paclitaxel administered in ABI-007 is less toxic than

TAXOL or the TAXOL vehicle administered alone. The LD_{50} and LD_{10} for ABI-007 were 447.4 and 371.5 mg/kg of paclitaxel, 7.53 and 5.13 mg/kg of paclitaxel in TAXOL, and 1325 and 794 mg/kg of the TAXOL vehicle (corresponds to a dose of 15.06 and 9.06 mg/kg TAXOL), respectively. In this study, the LD_{50} for ABI-007 was 59 times greater than TAXOL and 29 times greater than the TAXOL vehicle alone. Most deaths in the TAXOL and TAXOL vehicle treated groups occurred on day 0 which suggests the toxicity of TAXOL is due to an acute reaction to the vehicle. Such acute reactions are unlikely to occur in human patients treated with ABI-007 because of the difference in vehicles.

A pilot study was conducted to compare the LD_{50} of ABI-007 and TAXOL following daily (five days) intravenous administrations into mice. The results indicated that ABI-007 is less toxic than TAXOL. The LD_{50} of ABI-007 was 76.2 mg/kg of paclitaxel, compared to 8.07 mg/kg of paclitaxel in TAXOL. In this study, the LD_{50} for ABI-007 was 9.4 times higher than for TAXOL. All deaths (11) for TAXOL-treated animals occurred on days 0 - 4 after start of treatment while deaths for ABI-007-treated animals (5) occurred on days 6 or 9 after the start of treatment. The results of this study suggest that the ABI-007 is less toxic than TAXOL when administered in multiple doses at daily intervals. Human patients will initially receive ABI-007 infusion over a period of approximately 30 minutes with 3-week intervals between treatments, rather than a 1 day interval as in the animal studies. Toxicity from treatment with ABI-007 is considerably less than treatment with TAXOL.

Example 41

Toxicology in Rats

A further study was conducted to determine the toxicity of ABI-007 following a single IV administration at approximately 1 mL/minute with a total volume of 5 mL/kg to 6 male and 6 female rats at each dose of 5, 9, 30, 90 and 120 mg/kg.

One half of the animals from each group were euthanized and necropsied on day 8. The remaining animals were necropsied on day 31. The results of the ABI-007 treated animals were compared to the results of normal saline and vehicle control groups, as well as to the results of animals treated with 5, 9 and 30 mg/kg TAXOL.

Thirteen rats died in the study. Twelve of the animals treated with 30 mg/kg TAXOL® and one treated with ABI-007 died on day 4. In comparison, all but one animal treated with ABI-007 at doses up to and including 120 mg/kg survived. One animal treated with 90 mg/kg ABI-007 had an unexplained death.

Animals were observed for overt manifestations of toxicity immediately following dosing, 1 hour and 4 hours post-administration and once daily for 7 or 30 days. All animals in each group appeared normal immediately post-administration. At 1 and 4 hours post-administration, rats in the TAXOL-treated groups were observed to have piloerection and staggering gaits.

This reaction was possibly due to the high alcohol content of the TAXOL vehicle. Animals treated with ABI-007 at doses up to and including 30 mg/kg were normal throughout the 7 day and 30 day observation periods. A few incidents of piloerection were reported in animals treated with 90 and 120 mg/kg of ABI-007.

Male rats in the 120 mg/kg ABI-007 treated group had reduced body weights at day 8 when compared to the control animals. The animals did recover some of the lost weight by the end of the study.

Hematology and serum chemistries were generally unremarkable in this study. The most notable findings were a tendency towards marginally high sodium concentrations and correspondingly low potassium concentrations in the animals treated with higher doses of ABI-007. Despite statistical significance when compared to controls, this high concentration of sodium was still within normal parameters. Animals treated with 120 mg/kg ABI-007 were reported to have a high BUN concentration on day 8, but values returned to normal by day 31.

At necropsy, significant lesions were observed in the male reproductive organs of animals dosed with 9, 30, 90 and 120 mg/kg ABI-007. These changes involved diffuse degeneration and necrosis of the testis. These changes were most prevalent in animals that received higher doses of ABI-007. Female rats did not demonstrate toxic effects from ABI-007 doses up to and including 120 mg/kg. ABI-007 was considerably less toxic than TAXOL. No TAXOL-treated animals survived at doses higher than 9 mg/kg.

With the exception of an incidental death at 90 mg/kg ABI-007, all animals survived doses to 120 mg/kg. Human patients should also experience less toxic effects with ABI-007 treatment than with TAXOL.

Example 42

Myelosuppression in Rats

Myelosuppression and other hemopoietic effects have been reported as adverse events after treatment with TAXOL. A pilot study was designed to compare the effects of ABI-007 with TAXOL in rats after a single intravenous injection. The effects of both the ABI-007 and TAXOL carrier vehicles were also tested. Both ABI-007 and TAXOL were tested at a single dose of 5 mg/kg paclitaxel while the carrier vehicles were tested individually at the respective concentrations used to suspend 5 mg/kg of paclitaxel. Therefore, 766 mg/kg of TAXOL vehicle and 50 mg/kg of ABI-007 vehicle were administered for these treatments. Four male rats were treated per test group by infusion over 3 hours. Changes in body weight and white blood cell counts were monitored for 14 days post treatment to evaluate the hemopoietic effects.

ABI-007 produced significantly less ($P < 0.05$) myelosuppression than TAXOL as determined by white cell counts at days 1 and 7 and a highly significant ($P<0.01$) reduction in white cell counts at 10. The recovery period from the white blood cell suppression after a 5 mg/kg dose of paclitaxel was only about 7 days for ABI-007 but was about 14 days for TAXOL. ABI-007 also showed significantly less decreases in weight at days 1 and 10 than TAXOL. The TAXOL vehicle

(Cremophor/ethanol) had a very strong neutropenic effect. The TAXOL vehicle decreased WBCs for days 1 and 3 ($P<0.01$) when compared to the ABI-007 vehicle and also significantly decreased WBCs on day 1 when compared to ABI-007 ($P<0.05$). Significant decreases in body weights ($P<0.05$) were also observed for the TAXOL vehicle when compared to both ABI-007 and its vehicle.

This suggests that the TAXOL vehicle may be the major cause of myelosuppression from TAXOL. However, the ABI-007 vehicle (human albumin) showed no significant myelosuppression in this study. Therefore, neutropenia from treatment in human patients with ABI-007 should be considerably less than treatment than with TAXOL.

Example 43

Canine Toxicology

The animals in all three treatment groups exhibited a common syndrome of gastrointestinal symptoms, edema, vasculitis, and organ and tissue abnormalities. These toxic effects, indicate that the animals were reacting to human albumin, a constituent of the formulation common to all three groups. That ABI-007-treated animals had more severe and numerous clinical symptoms suggests that the cytotoxic effects of paclitaxel in the ABI-007 exacerbated the effects of the human albumin.

The effects seen in ABI-007-treated animals other than the common syndrome, are consistent with changes seen in other paclitaxel studies. However, the toxicity of ABI-007 at 175 mg/m^2 was not established in this study.

Example 44

Phase I Clinical study of Intravenous ABI-007 in Solid Tumors:

In a Phase I, open-label, dose-ranging trial of ABI-007 therapy in patients with advanced solid tumors conducted at M.D. Anderson Cancer Center, more than 80 courses of ABI-007 were administered intravenously (IV) over 30 minutes, every 3

weeks to 19 patients. The total number of doses of ABI-007 administered was 83. The maximum dose administered was 375 mg/m^2, which was administered to six patients (between 25-85 minutes). The maximally tolerated dose was determined to be 300 mg/m^2, which was also administered to six patients (between 27-60 minutes).

The following data for the Phase I study is preliminary, and is representative of all 19 evaluable patients entered into the study; however, data is still outstanding on three patients, who are currently receiving study drug. The Phase I study has been closed for enrollment and the maximum tolerated dose (MTD) for ABI-007 has been established to be 300 mg/m^2.

Patient Demographics: *Of the 19 enrolled patients,* 3 (15.8%) were male and 16 (84.2%) were female. Median age was 51 years (range: 34-83 years). The majority of patients were white 18 (94.7%). One patient was Hispanic (5.3%).

Patient Diagnoses and Condition: The primary diagnoses for the 19 patients enrolled were as follows: Six (31.6%) patients had melanoma, 12 (63.2%) had breast cancer and 1 (5.3%) had a primary diagnosis of unknown origin. Histology showed 11 (57.9%) patients diagnosed with invasive ductal carcinoma; 6 (31.6%) patients had malignant melanoma; 1 (5.3%) patient diagnosed with invasive lobular carcinoma, and 1 (5.3%) patient with Paget's disease of the nipple. At baseline all 19 patients reported having a Zubrod performance status score of 2 or less. Eleven (57.9%) patients reported having a Zubrod score of 1. Four (21.1%) patients reported a score of 0, and 4 (21.1%) patients reported a score of 2.

Baseline Physical Examination and Labs: The majority of patients had an abnormal physical examination (p.e.) at baseline. Sixteen (84.2%) patients had abnormal physical exams, 2 (10.5%) patients had a normal p.e., and baseline p.e. data was missing on 1 (5.3%) patient. The median weight at baseline was 70.7 kg (range: 46.9-100.7). The median systolic blood pressure was 118 (range: 96-180) and diastolic was 74 (range: 50-96). Median laboratory values at baseline versus median laboratory values during all dosing cycles of the study are displayed below:

Lab	Baseline Median Lab Values	Median Values Throughout the Study
HGB	11.6 (range: 8-14.3)	11.2 (range: 7.4-14.6)
	243.5 (range: 154-509)	253 (range: 25-585)
WBC	5.9 (range: 3.2-11.9)	4 (range: 0.4-32)
NEUTS (%)	69 (range: 59-84)	63.9 (range: 3-94)

Prior Therapy: All 19 patients had received prior therapy. Nineteen (100%) patients had prior chemotherapy; 6 (31.6%) patients had prior hormonotherapy; 7 (36.8%) patients had prior immunotherapy; 17 (89.5%) patients had prior radiotherapy; and 17 (89.5%) patients had prior surgery.

Relevant Prior Chemotherapy: All 19 patients had received prior chemotherapy. Twelve (63.2%) had previously received doxorubicin; 8 (42.1%) had received Taxol, and 2 (10.5%) had received Herceptin. Patients had received many other commonly used drugs.

Dose Escalation: Four dose levels were utilized in this study. The starting dose level of ABI-007 was 135 mg/m^2. Four patients were treated at this dose. The dose was escalated by 50% to 200 mg/m^2, at which 3 patients were treated. The dose was further escalated by 50% to 300 mg/m^2 at which 6 patients were treated. The final escalation in dose was by 25% to 375 mg/m^2 at which 6 patients were treated. It was eventually determined that 300 mg/m^2 was the maximum tolerated dose (MTD). At dose levels of 135mg/m^2 and less than 200 mg/m^2, there were no adverse events of myelosuppression or peripheral neuropathy.

Example 45

Intravenous ABI-007: Safety and Toxicity

ABI-007 was well tolerated at doses up to 300 mg/m^2, which were delivered over a period of 27-60 minutes. Most adverse events were Grade 1 or 2 (93%), required no action (83%) and were resolved (78%). No deaths occurred during the study. Five patients had reductions in their study drug dosages, changes in their

frequency, interruptions in dosing and/or discontinuation of study drug during the study. All five patients had these changes to their study drug regimen as the result of the development of unacceptable toxicities. Of these five patients, four reported sensory changes, three reported vision-related toxicities, two experienced asthenia, and two patients experienced thrombocytopenia. All other patients receiving a dose from 135 mg/m^2 to 300 mg/m^2 showed no such evidence of thrombocytopenia.

The most common (≥3% of all 377 reported events) adverse events were asthenia (13.1%), nausea (8.8%), fever (6.8%), sensory changes (6.1%; sensory changes included: sensory loss, tingling, paresthesia, and peripheral neuropathy), arthralgia (5.7%), stomatitis (5.1%), myalgia and headache (both at 4.5%), diarrhea, rash, vomiting and visual disturbances (all at 4.3%; visual disturbances included: vision abnormalities (2.0%), dry eyes (1.4%), and keratitis (0.9%)), and hypertonia (3.4%).

The adverse events reported by the majority of patients (given as % of patients) were asthenia (84.21%), nausea (68.42%), sensory changes (63.16%), rash (57.89%), fever, myalgia, and stomatitis (all at 52.63%), headache (42.11%), diarrhea and vomiting (both at 36.84%), arthralgia (31.58%) and hypertonia (26.32%). Sensory changes and visual disturbances were the only toxicities which appeared to be dose-related. Sixteen out of the 23 reported sensory toxicities and 6 out of the 17 vision-related toxicities occurred in patients receiving ≥ 300 mg/m^2.

Grade 3 Toxicities: There were 27 occurrences of Grade 3 reported toxicities, including: fatigue and sensory changes, both at 5 (19.2%), stomatitis 3 (11.5%), diarrhea and keratitis, both at 2 (7.7%), bone pain, constipation, cough, dyspnea, hypoxia, infection, nausea, pleural effusion, skin reaction, and vomiting all were reported as single occurrences (3.8%). (% of total is reflective of the number of occurrences out of the total 27 Grade 3 reported toxicities.)

Nineteen out of the 27 (70.4%) reported Grade 3 toxicities were rated potentially study drug related. Only 4 (21%) out of the 19 remain unresolved; one fatigue and three cases of sensory changes. These four cases occurred in three

different patients and action (study drug dose reduction) was required for only one case of sensory toxicity.

Hematologic Toxicities: There was a total of 13 (68.4%) patients that reported an Absolute Neutrophil Count (ANC)< 2000 cells/mm^3. Of the total 13 patients, 2 (15%) were at a dose level of 135 mg/m^2, 1 (8%) patient was at dose level 200 mg/m^2, 5 (38%) patients were at dose level 300 mg/m^2, and 5 (38%) patients with ANC < 2000 cells/mm^3 were at the dose level of 375 mg/m^2. Two patients, at dosing level 375 mg/m^2, resolved without any action taken; however, on their following cycle both patients under went a dose reduction due to thrombocytopenia and one case of Grade 3 skin reaction, 198.86 mg/m^2 and 361.3 mg/m^2, respectively.

Two (10.5%) patients experienced thrombocytopenia, both dosed at 375 mg/m^2. The first patient resolved when dosing level was reduced to 198.86 mg/m^2. The second patient resolved without any action taken; however, on the patient's next dosing cycle, study drug was administered at a slightly lower dose of 361.38 mg/m^2. All other patients receiving a dose from 135 mg/m^2 to 300 mg/m^2 showed no such evidence of thrombocytopenia.

Sensory Toxicities: Sensory changes (including sensory loss, tingling, paresthesia, and peripheral neuropathy) were reported by 12 (63.16%) out of 19 patients. There were a total of 23 reported occurrences of sensory toxicities. Eight (34.8%) occurrences of sensory toxicity were reported as Grade 1 (including two patients with pre-existing condition at baseline). A total of 10 (43.5%) occurrences of sensory toxicity were rated as Grade 2 and 5 (21.7%) reported cases were rated as Grade 3. Two out of the 23 (8.7%) occurrences were pre-existing sensory toxicities experienced by two patients prior to study drug administration. These two patients went on to receive study drug and there were no reports of any sensory toxicities while receiving ABI-007. Five out of the 23 (21.7%) occurrences took place at dosing levels of 200 mg/m^2 to < 300 mg/m^2. Sixteen out of the 23 (70%) occurrences were at dosing levels ≥ 300 mg/m^2. Ten (43.5%) occurrences out of the 23 reported sensory toxicities occurred at dosing levels > 370 mg/m^2. Action was taken on only

four out of the total 23 sensory reported toxicities. Two patients discontinued study drug for the remainder of the cycle, one patient was at dose level 375.28 mg/m^2 and the second patient was at 370.83 mg/m^2; however, they both resumed their following cycle at a dose reduced study drug level, 198.86 mg/m^2 and 300 mg/m^2, respectively. The third patient reported a Grade 3 sensory toxicity while at a dosing level of 301.07 mg/m^2. Patient continued on the next cycle at a dose reduced level of 198.93 mg/m^2. The fourth patient (dosing level 301.50 mg/m^2) experienced a Grade 2 sensory toxicity. Study dose level was reduced to 200 mg/m^2 for the following cycle.

Considering that 2 of the 12 patients included amongst those reporting sensory changes had pre-existing sensory toxicities prior to receiving the study drug and that these patients did not report any further sensory changes while on the study, it may be concluded that sensory changes occurred in 10 (52.6%) out of 19 patients while they were on the study drug. Grade 3 sensory toxicities occurred in 4 (21%) of 19 patients.

Ocular or Vision Toxicities: A total of 17 occurrences of visual disturbances, including decreased vision associated with burning sensation, foreign body sensation and photophobia were reported by 10 (52.6%) patients while receiving ABI-007. In the opinion of the Investigator, 10 (63%) of the 17 visual toxicities were possibly study drug related.

Six (35%) of the reported cases were classified as definitely study drug related. One (6%) occurrence rated as a Grade 2 was classified as not study drug related. Of the 16 occurrences that were possibly or definitely study drug related, 9 (56%) were rated Grade 1, 5 (31%) were Grade 2, and 2 (12.5%) were rated as Grade 3. Ten (58.8%) occurrences required no action to be taken. The single occurrence that was classified as not study drug related required treatment for symptoms only. Six (35%) occurrences required action. These six occurrences were among three patients. The first patient experienced three separate visual disturbances: blurred vision (Grade 2), keratitis (Grade 3) and another occurrence of blurred vision (Grade 1), all at dose level 375.28 mg/m^2. Patient's study drug regimen was interrupted;

however, the patient continued on the following cycle at a dose reduced level of 198.86 mg/m^2. The second patient requiring action, experienced one occurrence of Grade 2 abnormal vision and a Grade 3 keratitis. Dosing regimen of 370.83 mg/m^2 was interrupted; however, patient resumed dosing at a reduced level of 300 mg/m^2. The third patient had a Grade 2 occurrence of keratitis while receiving ABI-007 at a dosing level of 301.50 mg/m^2. Action taken was a dose reduction on the following cycle to 200 mg/m^2. These symptoms were resolved by aggressive lubrication and placement of collagen punctal plugs.

The incidence of adverse events for the above-described Phase 1 study are summarized in Table 5.

Table 5. Summary table of Toxicities

ADVERSE EVENT	Number (% of Total) of Occurrences	Number (% of Total) of Patients
Alopecia	10 (2.8%)	10 (52.6%)
Amblyopia	3 (0.8%)	2 (10.5%)
Anorexia	9 (2.5%)	8 (42.1%)
Anxiety	2 (0.6%)	2 (10.5%)
Arthralgia	20 (5.7%)	6 (31.6%)
Asthenia	46 (13.0%)	15 (78.9%)
Chills	6 (1.7%)	1 (5.3%)
Constipation	10 (2.8%)	9 (47.4%)
Convulsions	1 (0.3%)	1 (5.3%)
Cough, Increased	2 (0.6%)	1 (5.3%)
Cramps Leg	1 (0.3%)	1 (5.3%)
Depression	3 (0.8%)	3 (15.8%)
Diarrhea	15 (4.2%)	7 (36.8%)
Dyspepsia	3 (0.8%)	2 (10.5%)
Dyspnea	3 (0.8%)	3 (15.8%)
Dysuria	1 (0.3%)	1 (5.3%)
Ear Disorder	2 (0.6%)	2 (10.5%)
Edema	2 (0.6%)	2 (10.5%)
Effusion Pleural	1 (0.3%)	1 (5.3%)
Emotional Lability	1 (0.3%)	1 (5.3%)
Fever	24 (6.8%)	10 (52.6%)
Flu Syndrome	7 (2.0%)	3 (15.8%)
Headache	16 (4.5%)	8 (42.1%)
Hem Vaginal	1 (0.3%)	1 (5.3%)
Hypertonia	12 (3.4%)	5 (26.3%)

Hypokalem	1 (0.3%)	1 (5.3%)
Hypoxia	1 (0.3%)	1 (5.3%)
Infection	5 (1.4%)	5 (26.3%)
Infect Urin Tract	1 (0.3%)	1 (5.3%)
Infect Viral	1 (0.3%)	1 (5.3%)
Insomnia	9 (2.5%)	6 (31.6%)
LDH, Increased	1 (0.3%)	1 (5.3%)
Leukopenia	1 (0.3%)	1 (5.3%)
Liver Func Abnormality	1 (0.3%)	1 (5.3%)
Myalgia	16 (4.5%)	10 (52.6%)
Nail Disorder	1 (0.3%)	1 (5.3%)
Nausea	31 (8.8%)	13 (68.4%)
Pain	1 (0.3%)	1 (5.3%)
Pain, Abdominal	2 (0.6%)	2 (10.5%)
Pain Bone	4 (1.1%)	3 (15.8%)
Pain Chest	1 (0.3%)	1 (5.3%)
Pharyngitis	2 (0.6%)	2 (10.5%)
Polyuria	2 (0.6%)	2 (10.5%)
Rash	15 (4.2%)	11 (57.9%)
Rhinitis	3 (0.8%)	2 (10.5%)
Sensory Changes	23 (6.0%)	12 (63.2%)
Speech Disorder	1 (0.3%)	1 (5.3%)
Stomatitis	18 (5.1%)	10 (52.6%)
Syncope	1 (0.3%)	1 (5.3%)
Thirst	3 (0.8%)	3 (15.8%)
Vasodilation	1 (0.3%)	1 (5.3%)
Visual Disturbance	17 (4.5%)	10 (52.6%)
Vomiting	15 (4.2%)	7 (36.8%)

Example 46

Comparison of Toxicities of ABI-007, TAXOL and TAXOTERE

Sensory toxicities (including sensory loss, tingling, paresthesia, and peripheral neuropathy) with ABI-007 occurred in 52.6% of patients while they were on the study drug. In comparison, peripheral neuropathy occurred in 70% of patients receiving TAXOL and neurosensory toxicities occurred in 56.8% of patients receiving TAXOTERE.

No hypersensitivity reactions were observed with ABI-007, whereas hypersensitivity was experienced in 36% of patients receiving TAXOL, and 17.6% of patients receiving TAXOTERE.

These findings suggest that at equivalent or higher doses of ABI-007 in this limited population, a much lower incidence of toxicity is observed in the ABI-007 group.

The MTD for ABI-007 was established at 300 mg/m^2. Preliminary, unaudited data from the Phase I trial of ABI-007 (given at 135-375 mg/m^2/30 min) indicate a lower incidence of adverse events than seen with the published information on TAXOL (given at 175 mg/m^2/3hr) and TAXOTERE (given at 100 mg/m^2/1 hr).

ABI-007 indicates 68% of patients reporting neutropenia at < 2000 cells/mm^3, compared to TAXOL (90%) and TAXOTERE indicating 98.5%. ABI-007 indicates 32% of patients (4 out of 6 patients at dose level 375 mg/m^2) experiencing neutropenia at < 500 cells/mm^3 compared to TAXOL (28%) and TAXOTERE (85.9%).

Example 47

Clinical Pharmacokinetics of ABI-007

In Phase I of this trial, 19 patients with solid tumor/breast cancer were assigned to four cohorts of at least 3 patients each. The starting ABI-007 dose at 135 mg/m^2 was administered to the first cohort via intravenous infusion at 3-week intervals. After a minimum of 3 patients in the first cohort had been treated with the starting dose infused over 180 minutes, the infusion duration was decreased to 30 minutes. The ABI-007 doses for the three remaining cohorts were 200, 300, and 375 mg/m^2, respectively, with each ABI-007 dose infused over a duration of approximately 30 minutes.

Sixteen of the 19 patients entered into the study contributed analyzable pharmacokinetic profiles. Following the termination of ABI-007 intravenous infusion in individual patients, the decline from maximum plasma concentration was biphasic. Pharmacokinetic analysis of individual patient profiles yielded pertinent pharmacokinetic parameters, which are summarized in Table 6.

Table6. Summary of Non-Compartment Pharmacokinetic Parameters Mean (% Coefficient of Variation) Values by Single Dose and Infusion

Dose	Infusion Duration	N	C_{max}	AUC_{inf}	Half-Life	CL	V_z
(mg/m^2)	(min)		(ng/mL)	(ng.h/mL)	(h)	($L/h/m^2$)	(L/m^2)
135	180	3	1392 (30)	5654 (42)	12.9 (60)	27.4 (45)	418 (32)
135	30	1	6100	6427	14.6	21.1	442
200	30	3	7757 (35)	9613 (20)	13.4 (67)	21.4 (21)	384 (64)
300	27 – 30	5	13520 (7)	17610 (21)	14.6 (14)	17.7 (22)	370 (23)
375	30 – 45	4	19350 (15)	35805 (40)	13.2 (12)	11.9 (42)	236 (54)

N Number of patients
C_{max} Maximum or peak concentration
AUC_{inf} Area under the whole blood/plasma concentration-time curve from time zero to time infinity
CL Total body clearance
V_z Volume of distribution

The paclitaxel AUC_{inf} value increased exponentially versus ABI-007 dose. This dose-dependent pharmacokinetic behavior was reflected by decreasing CL values as the ABI-007 dose was raised from 135 mg/m^2 to 375 mg/m^2. Similar findings have been reported Taxol.

Furthermore, based on a visual inspection the ABI-007 pharmacokinetic parameters obtained in the present study versus published pharmacokinetic parameters for Taxol, both products produced similar paclitaxel AUC_{inf}, half-life, and plasma clearance values at similar doses. However, ABI-007 could be infused intravenously over a shorter duration, i.e., 30 to 45 minutes compared to the intravenous infusion of Taxol, which normally takes 3 to 24 hours.

Protocol DM97-123 is an open non-randomized Phase I/II trial of ABI-007 in patients with solid tumor/breast cancer. In Phase I of this protocol, 19 patients were entered and were assigned to four cohorts of at least 3 patients each. The starting ABI-007 dose at 135 mg/m^2 was administered to the first cohort via intravenous infusion at 3-week intervals. After a minimum of 3 patients in the first cohort had been treated with the starting dose infused over 180 minutes, the infusion

duration was decreased to 30 minutes. The ABI-007 doses for the three remaining cohorts were 200, 300, and 375 mg/m^2, respectively, with each ABI-007 dose infused over a duration of approximately 30 minutes.

This preliminary clinical pharmacokinetic report summarizes the results of the pharmacokinetic analysis of the patients who contributed analyzable plasma drug concentration profiles.

Each cohort had a minimum of 3 patients. Each patient received ABI-007 via intravenous infusion at 3-week intervals. The ABI-007 infusion duration varied from 27 minutes to 180 minutes depending on the targeted ABI-007 dose.

Targeted ABI-007 Dose (mg/m^2)	Patient Number	Infusion Duration (min)
135	1, 2, 3	180
	4	30
200	5, 6, 7	30
300	8, 9, 11, 12, 13	27 – 30
375	15, 16, 17, 19	30 – 45

Whole blood/plasma samples were collected from each patient at baseline and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 14, 24 and 48 hours from the start of the first intravenous infusion of ABI-007. The whole blood/plasma samples were frozen and shipped on dry ice to Alta Analytical Laboratories, El Dorado Hills, California for assay of paclitaxel concentrations. A highly specific and sensitive LC/MS/MS assay was used in the analysis of whole blood/plasma samples. The limit of quantification of the assay was 5 ng/mL.

Plasma samples were obtained from all four patients assigned to receive the starting ABI-007 dose at 135 mg/m^2 and from the first patient in the second cohort assigned to receive ABI-007 dose at 200 mg/m^2. Whole blood samples were collected from the remaining patients.

Nineteen patients (16 females and 3 males) were enrolled. There were 18 Caucasians (95%) and 1 Hispanic (5%). The median age was 51 years (range: 34 – 83 years). Sixteen of the 19 enrolled patients contributed analyzable plasma drug concentration profiles. Of these 16 patients, 4 were assigned to receive ABI-007 at 135 mg/m^2, 3 were assigned to receive ABI-007 at 200 mg/m^2, 5 were assigned to receive ABI-007 at 300 mg/m^2, and 4 were assigned to receive ABI-007 at 375 mg/m^2.

Pharmacokinetic parameters were determined from each patient's whole blood/plasma paclitaxel concentration profile. The non-compartmental routine in WinNonlin was used in the analysis. The peak or maximum paclitaxel concentration (C_{max}) and the corresponding peak time (t_{max}) were observed values. The elimination constant (λ_z) was obtained by log-linear regression analysis of the terminal phase of the whole blood/plasma concentration versus time profile. The elimination half-life ($T_{1/2}$) was determined by taking the ratio of natural log of 2 and λ_z. The area under the curve from time zero to time infinity (AUC_{inf}) was obtained by summation of AUC_{last} (area under the curve from time zero to last measurable concentration, calculated by the linear trapezoidal rule) and AUC_{ext} (extrapolated area, estimated by taking the ratio between the last measurable concentration and λ_z). The dose-area relationship (i.e., total ABI-007 dose divided by AUC_{inf}) was used to determine total body clearance (CL). The volume of distribution (V_z) was determined by taking the ratio between CL and λ_z.

Descriptive statistics (mean, median, standard deviation, coefficient of variation, maximum and minimum) were computed for pertinent pharmacokinetic parameters by ABI-007 dose. No inferential statistics were performed due to the small sample size per cohort. However, linear regression analysis of AUC_{inf} versus dose was performed to gain an appreciation of pharmacokinetic linearity, if evident, for the dose range evaluated in this trial.

Sixteen of the 19 patients entered into the study contributed analyzable pharmacokinetic profiles. The individual patient plasma paclitaxel concentration

versus time profile, plotted in both Cartesian and semi-log coordinates. Figures showed the mean semi-log plasma paclitaxel concentration versus time plots for the four targeted ABI-007 doses. Both the individual patient plots and the mean profiles showed that plasma paclitaxel concentration climbed from the origin to maximum concentration (occurring at the termination of ABI-007 intravenous infusion). It is of interest to note the decline from maximum plasma paclitaxel concentration was biphasic.

Non-compartmental analysis of individual profiles yielded pertinent pharmacokinetic parameters for each patient. A listing of the individual pharmacokinetic parameter values (including C_{max}, T_{max}, $T_{1/2}$, AUC_{inf}, CL and V_z) was also provided by patient number. A summary of pharmacokinetic parameters by targeted ABI-007 dose is presented in the Table above.

The mean AUC_{inf} values increased more than proportionally versus ABI-007 dose. This non-linear pharmacokinetic behavior was also reflected by a decreasing CL term as the ABI-007 dose was raised.

The results of linear regression analysis of AUC_{inf} values versus dose showed that only 67% of the change in AUC_{inf} value could be explained by increases in ABI-007 dose. Similar dose dependent pharmacokinetic findings have been reported for Taxol (please see Taxol package insert).

Furthermore, based on a visual inspection the ABI-007 pharmacokinetic parameters obtained in the present study versus published pharmacokinetic parameters for Taxol, both products produced similar paclitaxel AUC_{inf}, half-life, and plasma clearance values at similar doses. However, the present study showed that ABI-007 could be infused intravenously over a shorter duration, i.e., 30 to 45 minutes compared intravenous infusion of Taxol, which normally takes 3 to 24 hours.

ABI-007, a Cremophor-free formulation of paclitaxel, offers the advantage of short intravenous infusion duration (e.g., 30 to 40 minutes) while achieving similar AUC_{inf} values as TAXOL.

Example 48

Summary of Data

All of the animal studies comparing ABI-007 to TAXOL have shown lower toxicity with ABI-007 and that it is the Cremophor EL vehicle, in part, that causes some of the toxicity associated with the TAXOL formulation of paclitaxel. These studies also indicate that paclitaxel in the ABI-007 formulation has the same efficacy in treating carcinomas as paclitaxel in the TAXOL formulation but with the advantage of lower toxicity as demonstrated by higher LD_{50} values for both single and multiple dose administrations and lower neutropenia. Another advantage appears to be that higher doses can be safely administered. A Phase I study in 19 patients with solid tumors established a maximum tolerated dose of 300 mg/m^2. There were no cases of hypersensitivity. Side effects at all doses up to 375 mg/m^2 included asthenia, nausea, fever, myalgia, headache, vomiting, diarrhea, arthralgia, rash, stomatitis, sensory changes, visual disturbances, constipation, and flu-like symptoms. Dose-related adverse events were sensory changes and vision disturbances, which occurred at doses $\geq$ 200 mg/m^2. The specific ocular complaints were described as decreased vision associated with burning sensation, foreign body sensation and photophobia. These symptoms were resolved with dose reduction and treatment. Also, the precautions and risks reported for TAXOL must be considered in the ABI-007 clinical trials. Adverse reactions that may occur are those that are attributed to the components of ABI-007 (paclitaxel and human albumin). These adverse reactions include neutropenia; thrombocytopenia; anemia; peripheral neuropathy; mucositis; myalgia/arthralgia; diarrhea; alopecia; elevation in bilirubin, alkaline phosphates and AST (SGOT); injection site reactions; and allergic reactions characterized primarily by fever and chills, rash, nausea, vomiting, tachycardia, hypotension, and the like. These reactions are described in the package inserts for TAXOL, TAXOTERE and for HUMAN ALBUMIN GRIFOLS®.

Patients who experience severe hypersensitivity reactions to ABI-007 should not be re-challenged with the drug. There are no studies on pregnant or nursing

women. These patients will be excluded from receiving this therapy. Females of childbearing age should take appropriate precautions to prevent pregnancy. In addition, one of the preclinical animal studies indicated degenerative lesions in the seminal vesicles and prostates of male rats treated with higher doses of paclitaxel in ABI-007. Therefore, male patients should be made aware of the possible risks to reproductive function.

As with all new pharmaceuticals in Phase I and II trials, there are possible, unknown risks. Therefore, both the treating physicians and patients must be vigilant to note and report any unusual occurrences or conditions. However, based on all the data presently available, ABI-007 is an effective pharmaceutical for treating patients with higher doses of paclitaxel than used previously and with less severe toxic effects.

Example 49

Phase I Clinical Study of Intra-arterial ABI-007 for Loco-regional solid tumors

In a Phase I, intra-arterial administration, open-label, dose-ranging trial of ABI-007 therapy in patients with advanced loco-regional solid tumors conducted at Insituto Nazionale Tumori, more than 200 courses of ABI-007 were administered intra-arterially over 30 minutes every 4 weeks to over 90 patients. The phase I study, open-label, in patients with loco-regional solid tumors is still ongoing. One hundred patients are planned.

The following data is preliminary and is representative of 74 patients (31 female and 43 male all of white race). Demographic data are reported in the attached tables.

The primary diagnosis of the 74 patients enrolled were as follows: 28 had hepatic-carcinoma (7 primary tumor, 21 secondary tumor) the remaining 46 had tumor in different districts: anal canal (12), bladder (6), head (16), neck (5), lung (2), pancreas (2), uterus (1), vulvae (1) e supernal (1).

Mean and median values concerning weight, height and age were reported and divided for type of tumors. Patients with liver carcinoma were treated with single doses between 150-450 mg for a maximum of three cycles. Patients with solid tumors received dose between 150-510 mg for a maximum of 4 cycles.

Example 50

Phase I Clinical Study of Intra-arterial ABI-007: Toxicity

Twenty-four patients with hepatic carcinoma and 45 patients with solid tumors showed 98 and 301 adverse events, respectively. Most events have been observed in the following: cutaneous (alopecia), systemic (asthenia and fever), gastro-intestinal (nausea, vomiting and stomatitis) and haematopoietic (anemia and leucopenia).

One patient with head carcinoma died during the study. The death occurred 6 days after the first cycle (dose 450 mg) due to heart failure; the patient was hospitalized and was diagnosed with hepatic failure and severe midollar deficiency. The Investigator judged the event possibly related to the study drug, but the current hepatic cirrhosis could have increased the toxicity. In patients with hepatic carcinoma, the intensity of the events was judged mild in 57.1% of the cases and moderate in the 42.8%; the 58.1% of the events do not require assistance and the 82.6% of the events recovered without sequelae for the patient. Then the investigator judged related to the study drug the 77.5% of the events (probable 35.7% and possible 41.8%). Concerning the solid tumors, the event intensity was mild in the 71.0%, moderate in the 26.5% and severe in the 2.3% of the cases; the 78% of the events do not require assistance and all the events recovered without sequelae in 67.4% of the cases. The drug correlation was of the 77.7% (probable in the 39.5% and possible in the 38.2 of the cases).

The events related to the study drug, have been judged in terms of toxicity according to WHO criteria. In patients with hepatic-carcinoma (primary tumor) the following intensity has been observed: grade 1 in 76.9% of the cases (23.1%

epigastralgia, 15.4% leucopenia e 15.4% alopecia), grade 2 in the 15.4% of the cases (alopecia). One patient showed a grade 3 stomatitis (7.7%).

In patients with hepatic-carcinoma (secondary tumor) the events with grade 1 are the 82.5% of the cases (15.8% leucopenia, 12.3% nausea , 12.3% anemia, and 10.5% asthenia), with grade 2 the 17.5% of the cases (12.3% alopecia). No patient reported superior grade toxicity. In case of solid tumor, the toxicity has been valuated for each type of tumor. In solid tumors the most common toxicity events (grade 1) were alopecia, asthenia, leucopenia, anemia and paresthesias. Alopecia, asthenia, leucopenia and stomatitis were the common events observed and judged of grade 2 toxicity. In patients with anal canal tumors were registered two cases of leucopenia classified of grade 3 and 4.

Laboratory values do not indicate changes clinically significant; the only relevant changes have already been documented as adverse events and if considered related to the study drug, have been valuated as toxicity events according to WHO toxicity grade.

Example 51

Phase I Clinical Study of Intra-arterial ABI-007: Efficacy

Seventy-two patients have been analysed in terms of efficacy data instead of 74 analysed for the toxicity data (Intention-to-treat), as the above-mentioned patient (with head carcinoma), died after the first cycle without performing any efficacy evaluation. The second patient (with vulvae carcinoma) was excluded from the analysis as lost to follow-up after the first cycle.

The response is defined according to WHO criteria, partial in 3 cases (11%), stable disease in 13 (46%) and progression in 12 (43%) patients with hepatic carcinoma (28).

Patients with head and neck carcinoma (20) showed a complete response in 2 cases (9.5%), partial in 14 cases (66.7%), stable in 2 cases (9.5%) and progression in 2 cases (9.5%).

Patients with anal canal carcinoma (12) showed a complete response in 1 case (8.3%), partial in 4 cases (33.4%), stable in 6 cases (50%) and progression in 1 case (8.3%).

Patients with bladder carcinoma (6) showed partial response in 5 cases (83.3%) and stable disease in 1 case (16.7%).

The remaining 6 patients (with lung, uterus, pancreatic and suprarenal carcinoma) showed partial response in 1 case (uterus), stable in 2 cases (lung, pancreas) and progression in the last 3 cases (lung, pancreas and suprarenal).

Example 52

Invention Compositions Comprising Devices for Delivery in Conjunction with Pharmacological Agents

Invention compositions, e.g., those containing drugs such as taxanes, are utilized in conjunction with devices for delivery in order to treat subjects in need of the medication or pharmacological agents. Devices contemplated for use with invention compositions include any type of tubing including polymeric tubings that may be utilized to administer the invention compositions or in general to administer drugs such as the taxanes or other antiproliferative drugs. Tubings of interest for use in the invention include catheter of any type, intravenous lines, arterial lines, intra-thecal lines, intracranial lines, catheters or tubing that may be guided by suitable means to any location within the subject, e.g., to the site of a stenotic blood vessel such as coronary artery or other artery or vein. Such tubings may also have the capability to carry baloons or stents that are useful for treatment of local narrowing, stenosis, restenosis, plaques including atherosclerotic plaques, thrombotic lesions, sites of hyperplasia, aneurysms or weakness in blood vessels.

Devices such as stents are also contemplated for use in combination with invention compositions. Stents may be fabricated from organic or inorganic materials, polymeric materials or metals. Invention compositions contemplate the combination of the invention pharmacological agents and devices mentioned herein.

Combination devices such as those comprising tubings along with baloons, stents, devices for local injection (e.g., into the lumen, into the vessel wall, into the intima of the blood vessel, into the endothelial or sub-endothelial layer, into the smooth muscle layer of blood vessels) etc. are also contemplated in combination with invention compositions of pharmacological agents.

Invention compositions of pharmacological agents or in general drugs such as the taxanes or other antiproliferative drugs and any drug or drugs contemplated by the invention may be delivered by the devices described above either by flowing through the device, being impregnated or embedded or stored within or with the device, or being able to be released or delivered at a local site of interest by the device or delivered by the device to be systemically available in the subject (e.g., intravenous administration).

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 composition for *in vivo* delivery of a substantially water insoluble pharmacologically active agent to a subject in need thereof, said composition comprising the product prepared by subjecting a mixture comprising:

an organic phase containing said pharmacologically active agent dispersed therein, and

aqueous medium containing biocompatible polymer,

wherein said mixture optionally contains substantially no surfactants,

to high shear conditions in a high pressure homogenizer at a pressure in the range of about 100 up to 100,000 psi.

2. A composition according to claim 1 wherein said organic phase is removed from said mixture.

3. A composition according to claim 1 wherein said aqueous phase is removed from said mixture.

4. A composition according to claim 1 wherein said pharmacologically active agent is paclitaxel and said biocompatible polymer is albumin.

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

6. A composition according to claim 5 wherein said particles have an average diameter of less than 1 micron.

7. A composition according to claim 5 wherein said particles have an average diameter of less than 200 nm.

8. A composition according to claim 7 wherein said mixture is sterile filtered.

9. A composition according to claim 5 wherein said particles are amorphous, crystalline, or a mixture thereof.

10. A composition according to claim 9 wherein said particles are substantially amorphous.

11. A composition for *in vivo* delivery of a substantially water insoluble pharmacologically active agent to a subject in need thereof, said composition comprising the product prepared according to the method comprising:
subjecting a mixture comprising:
an organic phase containing said pharmacologically active agent dispersed therein, wherein said organic phase comprises a substantially water immiscible organic solvent and optionally a water soluble organic solvent, and
aqueous medium containing biocompatible polymer,
wherein said mixture optionally contains substantially no surfactants,
to high shear conditions in a high pressure homogenizer at a pressure in the range of about 100 up to 100,000 psi.

12. A drug delivery system comprising particles of a solid or liquid, substantially water insoluble pharmacologically active agent, coated with protein,
wherein said protein coating has free protein associated therewith,
wherein a portion of said pharmacologically active agent is contained within said protein coating and a portion of said pharmacologically active agent is associated with said free protein, and
wherein the average diameter of said particles is no greater than about 1 micron.

13. A drug delivery system according to claim 12 wherein the average diameter of said particles is less than 200 nm.

14. A drug delivery system according to claim 13 wherein said system is sterile filtered.

15. A drug delivery system according to claim 12 wherein said particles are amorphous, crystalline, or a mixture thereof.

16. A drug delivery system according to claim 15 wherein said particles are substantially amorphous.

17. A drug delivery system according to claim 12 wherein said protein shell is suspended in a biocompatible aqueous liquid.

18. A method for the delivery of substantially water insoluble pharmaceutical agents to a subject, said method comprising administering to said subject an effective amount of composition prepared according to the method comprising subjecting a mixture comprising:

an organic phase containing said pharmacologically active agents dispersed therein, and

aqueous medium containing biocompatible polymer,

wherein said mixture optionally contains substantially no surfactants,

to high shear conditions in a high pressure homogenized at a pressure in the range of about 100 up to 100,000 psi.

19. A method for the delivery of substantially water insoluble pharmaceutical agents to a subject, said method comprising administering to said subject an effective amount of composition according to claim 1.

20. A method for the delivery of substantially water insoluble pharmaceutical agents to a subject, said method comprising administering to said subject an effective amount of said pharmaceutical agent as part of a drug delivery system according to claim 12.

21. A method to reduce the myelosuppressive effect of pharmaceutical agents, said method comprising administering an effective amount of said pharmaceutical agent as part of a drug delivery system according to claim 12.

22. A method according to claim 21 wherein said pharmaceutical agent is paclitaxel and said protein is albumin.

23. A method for eliminating cancer cells with a cremaphor free oncolytic comprising particles of an antineoplastic coated with protein,

wherein said protein coating has free protein associated therewith,

wherein a portion of said antineoplastic is contained within said protein coating and a portion of said antineoplastic is associated with said free protein, and

wherein the average diameter of said particles is no greater than about 1 micron.

24. A method according to claim 23 wherein the average diameter of said particles is less than 200 nm.

25. A method according to claim 24 wherein said system is sterile filtered.

26. A method according to claim 23 wherein said particles are amorphous, crystalline, or a mixture thereof.

27. A method according to claim 26 wherein said particles are substantially amorphous.

28. A method according to claim 23 wherein said antineoplastic is paclitaxel and said protein is albumin.

29. Bioprotected particles of a substantially water insoluble pharmacologically active agent coated with protein,

wherein said particles are surrounded by free protein associated therewith,

wherein a portion of said pharmacologically active agent is contained within said protein coating and a portion of said pharmacologically active agent is associated with said free protein surrounding said protein coating, and

wherein the average diameter of said particles is no greater than about 1 micron.

30. Bioprotected particles according to claim 29 wherein the average diameter of said particles is less than 200 nm.

31. Bioprotected particles according to claim 30 wherein said particles are sterile filtered.

32. Bioprotected particles according to claim 29 wherein said particles are amorphous, crystalline, or a mixture thereof.

33. Bioprotected particles according to claim 32 wherein said particles are substantially amorphous.

34. Bioprotected particles according to claim 29 wherein said pharmacologically active agent is paclitaxel and said protein is albumin.

35. An article comprising an intravenous catheter containing a composition according to claim 1.

36. An article according to claim 35 wherein said composition comprises paclitaxel incorporated into an albumin-based delivery system.

37. A method for reducing liver sequestration of pharmaceutical agents, said method comprising administering an effective amount of said pharmaceutical agent as part of a drug delivery system according to claim 12.

38. A method according to claim 37 wherein said pharmaceutical agent is paclitaxel and said protein is albumin.

39. A method for the administration of paclitaxel to a patient in need thereof employing a dosing solution containing >1 mg/ml of paclitaxel, said method comprising administering said paclitaxel as part of a drug delivery system according to claim 12.

40. A method for the administration of paclitaxel to a patient in need thereof employing a total infusion volume for each effective dose of <300 ml of paclitaxel-containing medium, said method comprising administering said paclitaxel as part of a drug delivery system according to claim 12.

41. A method for the rapid administration of paclitaxel to a patient in need thereof, said method comprising administering said paclitaxel as part of a drug delivery system according to claim 12.

42. A method of administering a pharmacologically active agent which must be administered in multiple doses, said method comprising administering the composition of claim 1 containing the pharmacologically active agent over a reduced cycle time.

43. A method of reducing the myelosuppressive effects of a pharmacologically active agent administered to a patient in need thereof, said method comprising administering the composition of claim 1 containing the pharmacologically active agent over a reduced cycle time.

44. A method of reducing the neurotoxic effects of a pharmacologically active agent administered to a patient in need thereof, said method comprising administering the composition of claim 1 containing the pharmacologically active agent over a reduced cycle time.

45. A method of administering pharmacologically active agent(s) to a patient having a disease capable of treatment by the pharmacologically active agent(s), said method comprising administering to the patient the composition of claim 1 containing the pharmacologically active agent(s).

46. The method of claim 45, wherein the disease is a proliferative disease and the pharmacologically active agent(s) comprises an anti-neoplastic agent.

47. The method of claim 45, wherein the disease is a cancer treatable by systemic administration of pharmacologically active agent(s), the administration is intravenous, and the pharmacologically active agent(s) comprises an anti-neoplastic agent.

48. The method of claim 45, wherein the disease is a cancer treatable by systemic administration of pharmacologically active agent(s), the administration is intraarterial, and the pharmacologically active agent(s) comprises an anti-neoplastic agent.

49. The method of claim 45, wherein conventional administration of the pharmacologically active agent(s) requires corticosteroid premedication, and wherein the administration of the composition is done without the use of corticosteroid premedication.

50. The method of claim 49, wherein the administration of the composition is done in combination with administration of biochemotherapy agent(s).

51. The method of claim 45, wherein conventional administration of a predefined dose regimen of the pharmacologically active agent(s) requires further

administration of a cytokine, and wherein the administration of the composition is done without the use of any cytokines.

52. A method of delivering pharmacologically active agent(s) to a localized area of a patient for sustained release of the pharmacologically active agent over an extended period of time, said method comprising administering to the patient the composition of claim 1 containing the pharmacologically active agent(s), wherein the composition has been dispersed within a matrix of suitable biocompatible material prior to administration to the patient.

53. A method of orally administering pharmacologically active agent(s) to a patient in need thereof, said method comprising orally administering the composition of claim 1 containing the pharmacologically active agent(s) in combination with intestinal cell efflux inhibitor(s).

54. A method of administering a combination of suitable pharmacologically active agent(s) to a patient in need thereof, said method comprising administering to the patient 25-75% of the conventionally effective dosage level of each of the suitable pharmacologically active agent(s) in the composition of claim 1.

55. A composition for *in vivo* delivery of a substantially water insoluble pharmacologically active agent to a subject in need thereof, said composition comprising said pharmacologically active agent as particles whose average diameter is no greater than about 1 micron.

56. The composition of claim 55, wherein said particles are substantially amorphous.

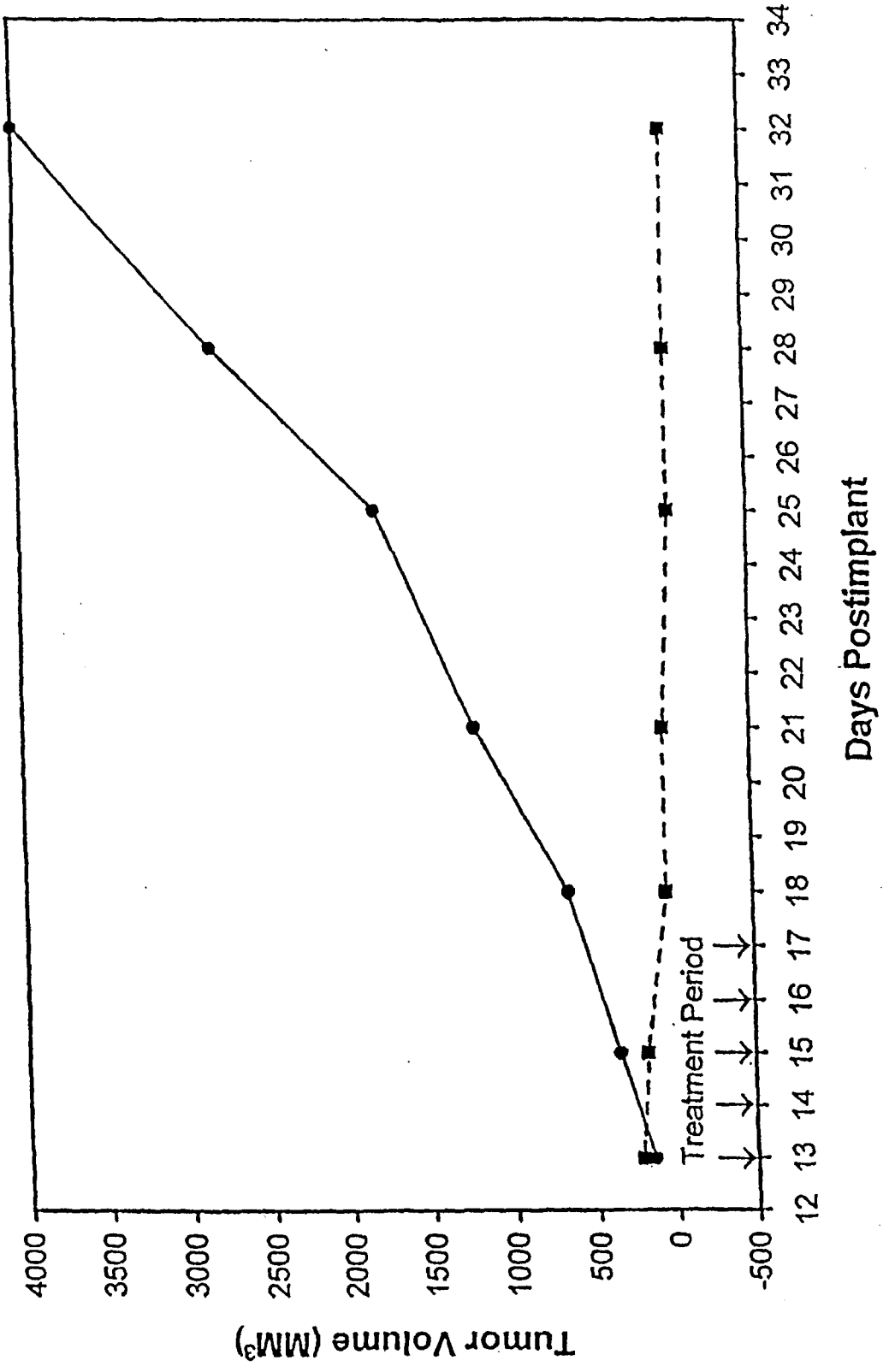
Tumor Volume (MM^3)
4000
3500
3000
2500
2000
1500
1000
500
0
-500
12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
Days Postimplant
Treatment Period

Figure 1

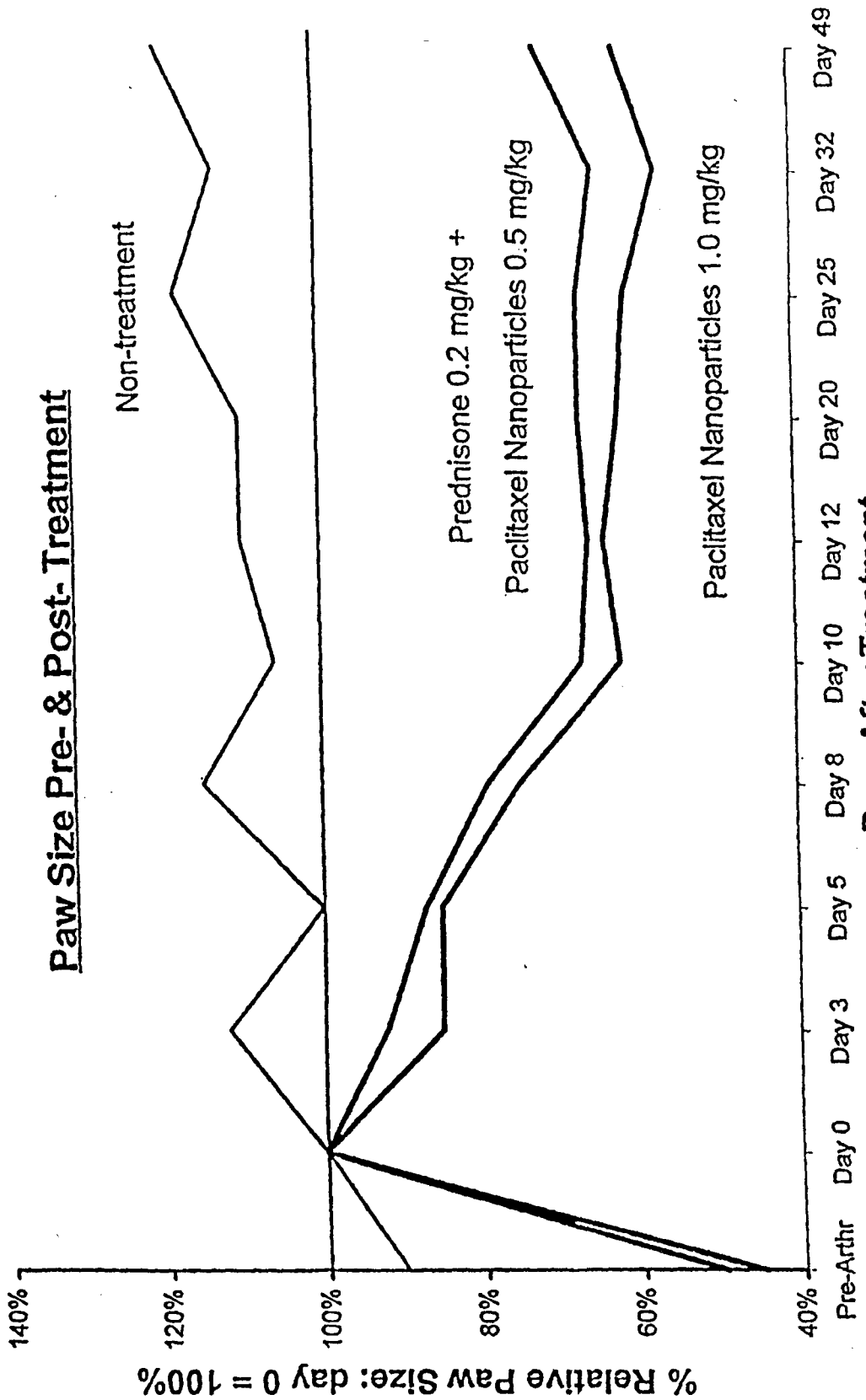
Paw Size Pre- & Post- Treatment
Non-treatment
Prednisone 0.2 mg/kg +
Paclitaxel Nanoparticles 0.5 mg/kg
Paclitaxel Nanoparticles 1.0 mg/kg
% Relative Paw Size: day 0 = 100%
140%
120%
100%
80%
60%
40%
Pre-Arthr
Day 0
Day 3
Day 5
Day 8
Day 10
Day 12
Day 20
Day 25
Day 32
Day 49
Days After Treatment

Figure 2