IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

ÉLAN PHARMACEUTICAL INTERNATIONAL LIMITED	)	
Plaintiff	)	C. A. No. _____
v.	)	
	)	**JURY TRIAL DEMANDED**
ABRAXIS BIOSCIENCE, INC.	)	
Defendant.	)	

COMPLAINT

Plaintiff Élan Pharmaceutical International Limited complains as follows against Defendant Abraxis BioScience, Inc.

THE PARTIES

1. Plaintiff Élan Pharmaceutical International Limited ("Élan") is a company organized and existing under the laws of Ireland, having a business address at Monksland, Athlone, Co. Westmeath, Ireland.

2. On information and belief, defendant Abraxis BioScience, Inc. ("Abraxis") is a corporation organized and existing under the laws of the State of Delaware, with its principal place of business at 11777 San Vincente Boulevard, Suite 550, Los Angeles, CA 90049.

JURISDICTION AND VENUE

3. This is an action for patent infringement under the patent laws of the United States, Title 35 of the United States Code. This Court has subject matter jurisdiction over this action under 28 U.S.C. §§ 1331 and 1338.

4. On information and belief, defendant Abraxis resides in this Judicial District, conducts business in this Judicial District, and has committed acts of patent infringement in this Judicial District including, *inter alia*, making, using, offering for sale, or selling an infringing product in this Judicial District.

5. On information and belief, defendant Abraxis has knowingly and actively contributed to infringement and/or has induced others to commit such acts of infringement in this Judicial District.

6. Venue is proper in this District pursuant to 28 U.S.C. §§ 1391 and 1400(b).

FACTUAL BACKGROUND

7. Plaintiff Élan is the owner by assignment of all right, title, and interest in and to United States Patent No. 5,399,363 ("the '363 patent") entitled "Surface Modified Anticancer Particles", which duly and legally issued in the names of Gary G. Liversidge, Elaine Liversidge, and Pramod P. Sarpotdar on March 21, 1995. A copy of the '363 patent is attached hereto as Exhibit A.

8. Plaintiff Élan is the owner by assignment of all right, title, and interest in and to United States Patent No. 5,834,025 ("the '025 patent") entitled "Reduction of Intravenously Administered Nanoparticulate-Formulation-Induced Adverse Physiological Reactions", which duly and legally issued in the names of Lawrence de Garavilla, Elaine

M. Liversidge, and Gary G. Liversidge on November 10, 1998. A copy of the '025 patent is attached hereto has Exhibit B.

9. On information and belief, on March 29, 2005 the United States Food and Drug Administration ("FDA") approved the sale of Abraxane® in the United States for treatment of metastatic breast cancer.

10. On information and belief, Abraxane® is a nanoparticle formulation of paclitaxel that is intravenously administered to patients for the treatment of breast cancer.

11. On information and belief, Abraxane® is a product owned, manufactured, offered for sale, or sold by the defendant Abraxis within this Judicial District.

COUNT I: INFRINGEMENT OF U.S. PATENT NO. 5,399,363

12. Élan realleges and incorporates by reference the allegations set forth in paragraphs 1-11 above.

13. On information and belief, defendant Abraxis has been, and currently is, directly and/or indirectly infringing the '363 patent by making, using, selling, and/or offering for sale Abraxane®; contributing to the use of Abraxane® by others; and/or inducing others to use Abraxane®.

14. On information and belief, defendant Abraxis's infringement of the '363 patent has been and continues to be willful and deliberate.

15. On information and belief, defendant Abraxis's infringement of the '363 patent will continue unless enjoined by this Court. As a result of the infringing conduct of Abraxis, Élan has suffered and will continue to suffer irreparable harm for which there is no adequate remedy at law. Accordingly, Élan is entitled to temporary, preliminary and/or permanent injunctive relief against such infringement pursuant to 35 U.S.C. § 283.

16. As a direct and proximate consequence of defendant Abraxis's infringement of the '363 patent, Élan has been damaged, and will be further damaged, and is entitled to be compensated for such damages pursuant to 35 U.S.C. § 284 in an amount not yet determinable, but which will be determined at trial.

COUNT II: INFRINGEMENT OF U.S. PATENT NO. 5,834,025

17. Élan realleges and incorporates by reference the allegations set forth in paragraphs 1-16 above.

18. On information and belief, defendant Abraxis has been, and currently is, directly and/or indirectly infringing the '025 patent by making, using, selling, and/or offering for sale Abraxane®; contributing to the use of Abraxane® by others; and/or inducing others to use Abraxane® in a manner that infringes the '025 patent.

19. On information and belief, defendant Abraxis's infringement of the '025 patent has been and continues to be willful and deliberate.

20. On information and belief, defendant Abraxis's infringement of the '025 patent will continue unless enjoined by this Court. As a result of the infringing conduct of Abraxis, Élan has suffered and will continue to suffer irreparable harm for which there is no adequate remedy at law. Accordingly, Élan is entitled to temporary, preliminary and/or permanent injunctive relief against such infringement pursuant to 35 U.S.C. § 283.

21. As a direct and proximate consequence of defendant Abraxis's infringement of the '025 patent, Élan has been damaged, and will be further damaged, and is entitled to be compensated for such damages pursuant to 35 U.S.C. § 284 in an amount not yet determinable, but which will be determined at trial.

PRAYER FOR RELIEF

WHEREFORE, Élan prays for entry of judgment against defendant Abraxis as follows:

That Abraxis has infringed the '363 and '025 patents under 35 U.S.C. §§ 271 *et seq.*;

That injunctions, preliminary and permanent, be issued by this Court restraining Abraxis, its respective officers, agents, servants, directors, and employees, and all persons in active concert or participation with each, from directly or indirectly infringing, or inducing or contributing to the infringement by others of the '363 and '025 patents;

That Abraxis be required to provide to Élan an accounting of all gains, profits, and advantages derived by Abraxis's infringement of the '363 and '025 patents, and that Élan be awarded damages adequate to compensate Élan for the wrongful infringing acts by Abraxis, in accordance with 35 U.S.C. § 284;

That the damages awarded to Élan be increased up to three times, in view of Abraxis's willful infringement, in accordance with 35 U.S.C. § 284;

That this case be declared to be exceptional in favor of Élan under 35 U.S.C. § 285, and that Élan be awarded its reasonable attorneys' fees and other expenses incurred in connection with this action;

That Élan be awarded its interest and costs of suit incurred in this action; and

That Élan be awarded such other and further relief as this Court may deem just and proper.

DEMAND FOR JURY TRIAL

Pursuant to Federal Rule of Civil Procedure 38(b) and Local Rule 38.1, Élan hereby demands a jury trial for all issues in this case that are properly subject to a jury trial.

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EXHIBIT A

US005399363A

United States Patent [19]

Liversidge et al.

[11] Patent Number: 5,399,363

[45] Date of Patent: Mar. 21, 1995

[54] **SURFACE MODIFIED ANTICANCER NANOPARTICLES**

[75] Inventors: **Gary G. Liversidge; Elaine Liversidge**, both of West Chester; **Pramod P. Sarpotdar**, Malvern, all of Pa.

[73] Assignee: **Eastman Kodak Company**, Rochester, N.Y.

[21] Appl. No.: **908,125**

[22] Filed: **Jul. 1, 1992**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 647,105, Jan. 25, 1991, Pat. No. 5,145,684.

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

[52] **U.S. Cl.** **424/490**; 424/489

[58] **Field of Search** 424/490, 487; 514/352

[56] **References Cited**

U.S. PATENT DOCUMENTS

2,671,750	3/1954	Macek	514/179
3,881,020	4/1975	Nakamura et al.	514/619
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4,269,821	5/1981	Kreuter et al.	424/489
4,540,602	9/1985	Motoyama	427/213.31
4,826,689	5/1989	Violanto	424/489
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5,049,322	9/1991	Devissaguet	424/490
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Remington's Pharmaceutical Sciences, 17th Edition, Chapter 20, Schott, H., "Colloidal Dispersions".

Goodman and Gilman, "The Pharmacological Basis of Therapeutics", Eighth Edition, pp. 68–69.

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[57] **ABSTRACT**

Dispersible particles consisting essentially of a crystalline anticancer agent having a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of less than about 1000 nm. Anticancer compositions comprising the particles exhibit reduced toxicity and/or enhanced efficacy, and can be administered by IV bolus injection.

17 Claims, No Drawings

SURFACE MODIFIED ANTICANCER NANOPARTICLES

CROSS REFERENCED TO RELATED APPLICATION

This application is a continuation-in-part of U.S. patent application Ser. No. 647,105, filed Jan. 25, 1991, now U.S. Pat. No. 5,145,684, the disclosure of which is hereby incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

1. Field of Invention

This invention relates to anticancer agents in the form of particles, to anticancer compositions comprising the particles, and to methods employing the particles.

2. Description of the Prior Art

The therapeutic index is a measure of how selective a drug is at producing its desired effects and can be defined as the ratio of the median lethal dose to the median effective dose, i.e., (LD_{50}/ED_{50}) (see Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, Eight Edition, p. 68–69). Virtually all anticancer agents have a low therapeutic index, e.g., less than about 1.0. Increasing the therapeutic index, e.g., by reducing toxicity or enhancing efficacy would provide more latitude to physicians in their duty of administering anticancer drugs to patients in need thereof. Consequently, methods to reduce toxicity and/or enhance efficacy of anticancer drugs and thus increase the therapeutic indices of such drugs would be of great value in the treatment of cancers.

In addition, poorly water-soluble drugs, such as poorly water-soluble anticancer agents, are not readily injectable via an intravenous (IV) bolus injection. The creation of injectable forms of poorly soluble drugs represents a formidable problem. It would be highly desirable to be able to provide poorly soluble drugs, such as poorly soluble anticancer agents, in an IV bolus injectable form.

SUMMARY OF THE INVENTION

We have discovered that anticancer compositions comprising anticancer agents in the form of surface modified nanoparticles exhibit reduced toxicity and/or enhanced efficacy.

More particularly, in accordance with this invention, there are provided particles consisting essentially of a crystalline anticancer agent having a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of less than about 1000 nm.

This invention further provides an anticancer composition comprising the above-described particles.

In another embodiment of the invention, there is provided a method of treating a mammal comprising administering to the mammal the above-described anticancer composition.

In yet another embodiment of the invention, there is provided a method of enhancing the efficacy and/or reducing the toxicity of an anticancer agent which includes the step of administering the agent in the form of the above-described particles.

It is an advantageous feature of this invention that anticancer compositions are provided exhibiting reduced toxicity.

It is another advantageous feature of this invention that anticancer compositions are provided exhibiting improved efficacy.

Yet another advantageous feature of this invention is that compositions are provided featuring poorly soluble anticancer agents that can be administered by IV bolus injection.

Still another advantageous feature of this invention is that compositions are provided containing poorly soluble anticancer agents exhibiting prolonged circulation in the blood pool after IV bolus injection.

Other advantageous features will become readily apparent upon reference to the following descriptions of preferred embodiments.

DESCRIPTION OF PREFERRED EMBODIMENTS

This invention is based partly on the discovery that surface modified anticancer nanoparticles exhibit reduced toxicity and/or enhanced efficacy. While the invention is described herein primarily in connection with its preferred class of drugs, i.e., anticancer agents including immunosuppressive agents, it is also useful in conjunction with poorly water soluble drugs, particularly those with low therapeutic indices, from other classes of drug substances.

The particles of this invention comprise an anticancer agent. The anticancer agent is present in one or more discrete crystalline phases. The crystalline phase differs from an amorphous, i.e., non-crystalline phase which results from conventional solvent precipitation techniques for the preparation of particles in the submicron size range, such as described in U.S. Pat. No. 4,826,689.

The invention can be practiced with a wide variety of anticancer agents. However, the anticancer agent must be poorly soluble and dispersible in at least one liquid medium. By "poorly soluble", it is meant that the drug substance has a solubility in the liquid dispersion medium, e.g., water, of less than about 10 mg/ml, and preferably, of less than 1 mg/ml at processing temperature, e.g., room temperature. The preferred liquid dispersion medium is water. However, the invention can be practiced with other liquid media in which the anticancer agent is dispersible including, for example, aqueous salt solutions, safflower oil, and solvents such as ethanol, t-butanol, hexane, and glycol. The pH of the aqueous dispersion media can be adjusted by techniques known in the art.

The anticancer agent preferably is selected from alkylating agents, antimetabolites, natural products, hormones and antagonists, and miscellaneous agents, such as radiosensitizers.

Examples of alkylating agents include alkylating agents having the bis-(2-chloroethyl)-amine group such as, for example, chlormethine, chlorambucile, melphalan, uramustine, mannomustine, extramustinephoshate, mechlore-thaminoxide, cyclophosphamide, ifosfamide, and trifosfamide;

alkylating agents having a substituted aziridine group such as, for example, tretamine, thiotepa, triaziquone and mitomycine;

alkylating agents of the alkyl sulfonate type, such as, for example, busulfan, piposulfan, and piposulfam;

alkylating N-alkyl-N-nitrosourea derivatives, such as, for example, carmustine, lomustine, semustine, or streptozotocine; and alkylating agents of the mitobronitole, dacarbazine and procarbazine type.

Examples of antimetabolites include folic acid analogs, such as, for example, methotrexate;

pyrimidine analogs such as, for example, fluorouracil, floxuridine, tegafur, cytarabine, idoxuridine, and flucytosine; and

purine derivatives such as, for example, mercaptopurine, thioguanine, azathioprine, tiamiprine, vidarabine, pentostatin, and puromycine.

Examples of natural products include vinca alkaloids, such as, for example, vinblastine and vincristine;

epipodophylotoxins, such as, for example, etoposide and teniposide;

antibiotics, such as, for example, adriamycine, daunomycine, doctinomycin, daunorubicin, doxorubicin, mithramycin, bleomycin, and mitomycin;

enzymes, such as, for example, L-asparaginase;

biological response modifiers, such as, for example, α-interferon;

camptothecin;

taxol; and

retinoids, such as retinoic acid.

Examples of hormones and antagonists include adrenocorticosteroids, such as, for example, prednisone;

progestins, such as, for example, hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate;

estrogens, such as, for example, diethylstilbestrol and ethinyl estradiol;

antiestrogens, such as, for example, tamoxifen;

androgens, such as, for example, testosterone propionate and fluoxymesterone;

antiandrogens, such as, for example, flutamide;

and gonadotropin-releasing hormone analogs, such as, for example leuprolide.

Examples of miscellaneous agents include radiosensitizers, such as, for example, 1,2,4-benzotriazin-3-amine 1,4-dioxide (SR 4889) and 1,2,4-benzotriazine-7-amine 1,4-dioxide (WIN 59075);

platinum coordination complexes such as cisplatin and carboplatin;

anthracenediones, such as, for example, mitoxantrone;

substituted ureas, such as, for example, hydroxyurea;

and adrenocortical suppressants, such as, for example, mitotane and aminoglutethimide.

In addition, the anticancer agent can be an immunosuppressive drug, such as, for example, cyclosporine, azathioprine, sulfasalazine, methoxsalen and thalidomide.

The anticancer agents useful in the practice of this invention are known compounds and/or can be prepared by techniques known in the art.

The anticancer agent can be used alone or in combination with one or more anticancer agents.

The particles of this invention contain an anticancer agent as described above having a surface modifier adsorbed on the surface thereof. Useful surface modifiers are believed to include those which physically adhere to the surface of the anticancer agent but do not chemically bond to the anticancer agent.

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and anionic surfactants. Representative examples of excipients include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens™, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP). Most of these excipients are described in detail in the *Handbook of Pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986. The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

Particularly preferred surface modifiers include polyvinylpyrrolidone, tyloxapol, polaxomers, such as Pluronic™ F68, F108 and F127, which are block copolymers of ethylene oxide and propylene oxide available from BASF, and poloxamines, such as Tetronic™ 908 (T908), which is a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine available from BASF, dextran, lecithin, Aerosol OT™ (AOT), which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanamid, Duponol™ P, which is a sodium lauryl sulfate, available from DuPont, Triton™ X-200, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas, Tween 20, 40, 60 and 80, which are polyoxyethylene sorbitan fatty acid esters, available from ICI Speciality Chemicals, Span 20, 40, 60 and 80, which are sorbitan esters of fatty acids, Arlacel 20, 40, 60 and 80, which are sorbitan esters of fatty acids, available from Hercules, Inc., Carbowax™ 3550 and 934, which are polyethylene glycols available from Union Carbide, Crodesta™ F-110, which is a mixture of sucrose stearate and sucrose distearate, available from Croda Inc., Crodesta SL-40, which is available from Croda, Inc., hexyldecyl trimethyl ammonium chloride (CTAC), bovine serum albumin and SA90HCO, which is $C_{18}H_{37}$ CH_2 (CON (CH_3) CH_2 $(CHOH)_4CH_2OH)_2$. Surface modifiers which have been found to be particularly useful include polyvinylpyrrolidone, Pluronic F-108, polyvinyl alcohol and gum acacia.

The surface modifier is adsorbed on the surface of the anticancer agent in an amount sufficient to maintain an effective average particle size of less than about 1000 nm. The surface modifier does not chemically react with the anticancer agent or itself. Furthermore, the individually adsorbed molecules of the surface modifier are essentially free of intermolecular crosslinkages.

As used herein, particle size refers to a number average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. By "an effective average particle size of less than about 1000 nm" it is meant that at least 90% of the particles have a number average particle size of less than about 1000 nm when measured by the above-noted tech-

niques. In particularly preferred embodiments of the invention, the effective average particle size is less than about 400 nm. In some embodiments of the invention, the effective average particle size is less than about 300 nm. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size of less than the effective average, e.g., 1000 nm. In particularly preferred embodiments, essentially all of the particles have a size less than 1000 nm.

Motoyama et al, U.S. Pat. No. 4,540,602 disclose that a solid drug can be pulverized in an aqueous solution of a water-soluble high molecular substance, and that as a result of such wet grinding, the drug is formed into finely divided particles ranging from 0.5 μm or less to 5 μm in diameter. However, there is no suggestion that particles having an average particle size of less than about 1 μm can be obtained. Attempts to reproduce the wet grinding procedures described by Motoyama et al resulted in particles having an average particle size of much greater than 1 μm.

The particles of this invention can be prepared by a method comprising the steps of dispersing an anticancer agent in a liquid dispersion medium and applying mechanical means in the presence of grinding media to reduce the particle size of the anticancer agent to an effective average particle size of less than about 1000 nm. The particles can be reduced in size in the presence of a surface modifier. Alternatively, the particles can be contacted with a surface modifier after attrition.

A general procedure for preparing the particles of this invention is set forth below. The anticancer agent selected is obtained commercially and/or prepared by techniques known in the art in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse anticancer agent selected be less than about 100 μm as determined by sieve analysis. If the coarse particle size of the anticancer agent is greater than about 100 μm, then it is preferred that the particles of the anticancer agent be reduced in size to less than 100 μm using a conventional milling method such as airjet or fragmentation milling.

The coarse anticancer agent selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the anticancer agent in the liquid medium can vary from about 0.1–60% and preferably is from 5–30% (w/w). It is preferred, but not essential, that the surface modifier be present in the premix. The concentration of the surface modifier can vary from about 0.1 to about 90% and preferably is 1–75%, more preferably 20–60%, by weight based on the total combined weight of the drug substance and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise.

The premix can be used directly by subjecting it to mechanical means to reduce the average particle size in the dispersion to less than 1000 nm. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the anticancer agent and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the particle size of the anticancer agent conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, a planetary mill, media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, i.e., the desired reduction in particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

The grinding media for the particle size reduction step can be selected from rigid media preferably spherical or particulate in form having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles of the invention with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical. However, zirconium oxide, such as 95% ZrO stabilized with magnesia, zirconium silicate, and glass grinding media provide particles having levels of contamination which are believed to be acceptable for the preparation of pharmaceutical compositions. Further, other media, such as stainless steel, titania, alumina, and 95% ZrO stabilized with yttrium, are expected to be useful. Preferred media have a density greater than about 2.5 g/cm^3.

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills, processing times of up to five days or longer may be required. On the other hand, processing times of less than 1 day (residence times of one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a temperature which does not significantly degrade the anticancer agent. Processing temperatures of less than about 30°–40° C. are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Processing pressures up to about 20 psi (1.4 kg/cm^2) are typical of media milling.

The surface modifier, if it was not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix above. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20–80 kHz for a time of about 1 to 120 seconds.

The relative amount of the anticancer agent and surface modifier can vary widely and the optimal amount of the surface modifier can depend, for example, upon the particular anticancer agent and surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, the surface area of the anticancer agent, etc. The surface modifier preferably is present in an amount of about 0.1–10 mg per square

meter surface area of the anticancer agent. The surface modifier can be present in an amount of 0.1–90%, preferably 0.5–80% and more preferably 1–60% by weight based on the total weight of the dry particle.

A simple screening process has been developed whereby compatible surface modifiers and anticancer agents can be selected which provide stable dispersions of the desired particles. First, coarse particles of an anticancer agent are dispersed in a liquid in which the anticancer agent is essentially insoluble, e.g., water at 2% (w/v) and milled for 120 hours in a roller mill under the following milling conditions:

Grinding vessel: 8 oz. (250 ml) glass jar
Available volume of grinding vessel: 250 ml
Media volume: 120 ml
Media type: 1.0 mm pre-cleaned zirconium oxide beads (distributed by Zircoa, Inc.)
Milling time: 120 hours
Slurry volume: 60 ml
RPM: 92
Room Temperature

The slurry is separated from the milling media by conventional means, e.g., by pouring the slurry out of the vessel, or by using a pipette. The separated slurry is then divided into aliquots and surface modifiers are added at a concentration of between 2 and 50% by weight based on the total combined weight of the anticancer agent and surface modifier. The dispersions are then sonicated (1 minute, 20 kHz) or vortexed using a multitubed vortexer for one minute, to disperse agglomerates and subjected to particle size analysis, e.g., by photon correlation spectroscopy (PCS) and/or by examination under an optical microscope (1000×magnification). If a stable dispersion is observed, then the process for preparing the particular anticancer agent surface modifier combination can be optimized in accordance with the teachings above. By stable it is meant that the dispersion exhibits no flocculation or particle agglomeration visible to the naked eye and, preferably, when viewed under the optical microscope at 1000×, at least 15 minutes, and preferably, at least two days or longer after preparation. In addition, preferred particles exhibit no flocculation or agglomeration when dispersed in 0.1N HCl and/or phosphate buffered saline, pH 7.4 (PBS) or rat plasma.

The resulting dispersion is stable and consists of the liquid dispersion medium and the above-described particles. The dispersion of surface modified anticancer agent nanoparticles can be spray coated onto sugar spheres or onto a pharmaceutical excipient in a fluid-bed spray coater by techniques well known in the art.

Anticancer pharmaceutical compositions according to this invention include the particles described above and a pharmaceutically acceptable carrier therefor. Suitable pharmaceutically acceptable carriers are well known to those skilled in the art. These include nontoxic physiologically acceptable carriers, adjuvants or vehicles for parenteral injection, for oral administration in solid or liquid form, for rectal administration, nasal administration, intramuscular administration, subcutaneous administration, and the like.

A method of treating a mammal in accordance with this invention comprises the step of administering to the mammal in need of treatment an effective amount of the above-described anticancer composition. The selected dosage level of the anticancer agent for treatment is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage can be readily determined by one skilled in the art and depends upon the particular anticancer agent, the desired therapeutic effect, the route of administration, the desired duration of treatment and other factors.

It is a particularly advantageous feature that the anticancer compositions of this invention exhibit reduced toxicity and/or enhanced efficacy as illustrated in the examples that follow. Further, the particles of this invention exhibit prolonged circulation in the blood pool.

Moreover, anticancer agents which heretofore could not be administered by injection, when prepared as nanoparticles and formulated in anticancer compositions according to this invention, can be effectively administered by injection, e.g., by an intravenous bolus injection.

The following examples further illustrate the invention.

EXAMPLES 1–4 Nanoparticulate Piposulfan

EXAMPLE 1

Piposulfan (purchased from Eastman Kodak) was milled in a mixture of 0.33% polyoxyethylene sorbitan monooleate, Tween 80, (ICI Americas, Inc., Wilmington, Del.) and 0.67% sorbitan monooleate, Span 80, (ICI) using 1 mm zirconium oxide beads for about 96 hours to produce particles approximately 240 nm in diameter. The final piposulfan concentration in the suspension was 10 mg/mL. The particles were stable to flocculation/aggregation in rat plasma.

Milling Conditions: A coarse suspension of piposulfan was prepared by adding 300 mg of the drug to a 4 oz. (120 mL) amber bottle which was previously filled with 60 mL of 1 mm precleaned zirconium oxide beads (Zircoa Inc., Solon, Ohio) and 30 mL of 1% Tween 80/Span 80 (1 to 2 ratio) solution. The surfactant solution was prepared by accurately weighing 333 mg of Tween 80 and 667 mg of Span 80 in a volumetric flask followed by addition of sterile water for injection to dissolve/disperse the surfactants. Sufficient quantity of water was added to make the final volume 100 mL. Zirconium oxide beads were cleaned by first rinsing in 1N sulfuric acid followed by several rinses with deionized water. The media was dried in a vacuum oven at about 100° C. overnight.

The sealed primary container was loaded into a secondary padded aluminium containment can to ensure a tight fit. It was milled on a roller mill (US Stoneware, Mawah, N.J.) at 144 RPM for about 96 hours. At the end of the milling time the slurry was separated from the media and particle size was measured using a PCS device. Stability of these particles to rat plasma was assessed by optical microscopy at 1000×magnification. The final pH of the formulation was 6.

Control A (unmilled), a coarse suspension containing 40 mg of bulk piposulfan was dispersed in water in the presence of 3% ethanol and 1% Tween 80. This suspension could not be injected IV.

Example 1 was evaluated for efficacy studies in female mice (avg. wt. 22 g) which were implanted with early stage Mammary Adenocarcinoma #16/C on day 0. The formulation was injected starting from day 1 for several days. The antitumor activity was assessed by monitoring tumor weight and comparing it to the control animals. The results were as follows:

Treatment	Route of Adm.*	Total Dose (mg/kg)	% Wt. Loss	T/C %	Log10 Cell Kill
Control	—	—	+5.5	—	—
Example 1	IV	356	−5.5	0	2.75
(243 nm)	IV	220	−5.5	2	2.75
	IV	137	−1.8	2	2.25
	IV	85	0	18	1.0
Control A	SC	800	−10.8	0	2.1

*Administration - IV Intravenous; SC Subcutaneous

Example 1 could be injected directly as 10 mg/ml suspension. There was no acute toxicity after injection of 78 mg/kg single dose.

T/C=Tumor weight in treated animals divided by the tumor weight of the control animals, reported as percent value. Lower value indicates better efficacy, 0% suggests cures. <10% is considered highly active, 10 to 42% is moderately active formulation. >42% is considered inactive.

Log Kill=(T−C)/3.32 (Td), where T is the time in days for the median tumor to reach 1000 mg mass in treated animals, C is the time in days for the median tumor to reach 1000 mg in control animals and Td is the tumor volume doubling time in days. Cures (tumor free animals) are excluded from (T−C) calculations.

Example 1 demonstrates that a composition of this invention exhibited reduced toxicity and enhanced efficacy compared to a prior art composition and could be administered by IV bolus injection.

EXAMPLES 2–4

The milling procedure described in Example 1 was repeated except that the ratio of Tween 80 to Span 80 was 2:1. The resulting average particle size was 297 nm.

The milling procedure described in Example 1 was repeated except that the ratio of Tween 80 to Span 80 was 1:1. The resulting average particle size was 380 nm.

The milling procedure described in Example 1 was repeated except that the surface modifier was a 1:1 ratio of Tween 60 and Span 60. The resulting average particle size was 301 nm.

Stable pipsulfan nanoparticles were also prepared using bovine serum albumin as the surface modifier.

EXAMPLES 5–7 Nanoparticulate Camptothecin

EXAMPLE 5

Approximately 60 mL of precleaned zirconium oxide beads (1 mm) were placed in a 120 mL wide mouth round amber bottle. To it was added 0.35 g of Tetronic 908 (BASF) followed by 0.35 g of Camptothecin (Sigma Chemicals, 95% pure). To the above mixture, 35 mL of water for injection (Abbott) was added. The bottle was sealed and mounted on a roller mill. Milling was effected by rotating the bottle at 100 RPM for 7 days.

At the end of milling, an aliquot (100 μL) was checked for particle size using a Malvern Zetasizer. The particles were determined to have an average particle size of 240 nm.

EXAMPLE 6

Example 5 was repeated except that the Tetronic 908 was replaced by polyvinyl alcohol (MW 30 to 70K). The final particle size was 256 nm.

EXAMPLE 7

Example 5 was repeated except that Tetronic 908 was replaced by gum acacia. The final particle size was 298 nm.

Nanocamptothecin formulations were evaluated for efficacy in two murine tumor models, i.e., Mammary Adenocarcinoma #16/C and Pancreatic Ductal Adenocarcinoma #03. The antitumor activity was assessed by monitoring tumor weight from experimental and control animals.

1. Efficacy Studies in Pancreatic Ductal Adenocarcinoma #03:

Example	Route	Dose mg/kg	Wt % Loss	Drug Deaths	T/C %
Control B	SC	60	−24.1	6/6	—
	SC	40.2	−21.8	5/5	—
	SC	26.7	−18.2	5/5	—
	SC	18	−10.9	1/5	62
6	IV	83.1	−16.7	1/4	14
	IV	78.2	−14.6	1/4	55
	IV	48.6	−8.3	0/4	0
	IV	24.3	−4.2	0/4	18
PVA Control	IV	—	+6.3	0/4	100
7	IV	93.5	−16.7	1/4	7
	IV	46.8	−14.6	0/4	17
	IV	23.4	−8.3	0/4	11
Cum Acacia Control	IV	—	0.0	0/4	60

The Control B formulation consisted of 1% coarse camptothecin in 3% ethanol, and 1% Tween 20. Control B could only be administered subcutaneously and even at the lowest SC dose (18 mg/kg) was inactive. Control B was toxic to 1/5 animals tested. In contrast, doses of the nanocamptothecin formulations of this invention ranging from 24–93 mg/kg were administered intravenously (IV) and were shown to be safe and efficacious.

2. Efficacy Studies in Mammary Adenocarcinoma #16/C Murine Tumor Model

Example	Route	Dose mg/kg	% Wt Loss	Drug Deaths	T/C %
Control B	SC	60	−23.5	5/5	—
	SC	30	−20.9	5/5	—
	SC	15	−18.3	3/5	14**
5	IV	65	−17.4	0/5	23
	IV	33	−18.7	1/5	33
	IV	16	−2.2	0/5	63
T908 Control	IV	—	+4.3	0/2	100
6	IV	65	−21.7	5/5	—
	IV	33	−15.7	0/5	100
PVA Control	IV	—	+4.3	0/2	100

**% T/C for the control animals was determined from the surviving animals, N = 2.

The T908 and PVA controls consisted of 1% aqueous solutions of the respective surface modifiers. The Control B was injected subcutaneously, and it was toxic at all doses tested. Efficacious doses of the nanocamptothecin formulations of this invention were administered intravenously.

3. Blood and Tumor Clearance

To determine if the increased efficacy was related to alterations in pharmacokinetic properties, blood clear-

ance and tumor distribution were studied in the Mammary adenocarcinoma #16/C murine tumor model.

Tumor bearing mice were injected via the tail vein with 10 mg/ml of camptothecin formulated as described in Examples 5 and 6 and a control which was 5 mg/ml camptothecin solubilized by the addition of 0.1N NaOH. At various times after injection, i.e., 5 min., 30 min., 60 min., 2 hrs., 4 hrs., 8 hrs., 16 hrs., 24 hrs. and 48 hrs., animals were euthanized and a blood sample was collected and the tumor excised. Concentrations of drug samples were quantified using HPLC. Results show that the compositions of this invention affect the clearance of the drug from the circulating pool of blood and the tumor.

	T½ Blood and Tumor	
Formulation	Blood	Tumor
Example 6	18 hrs.	>48 hrs.
Example 5	13 hrs.	>48 hrs.
Control	1.6 hrs.	13.5 hrs.

When formulated in accordance with this invention, the elimination half-life and the residence time of camptothecin in the tumor were prolonged significantly. It was concluded that pharmacokinetic parameters of the nanoparticulate formulation of camptothecin are directly related to the improved performance of the drug.

EXAMPLES 8–10 Nanoparticulate Etoposide

EXAMPLE 8

Example 5 was repeated except that 1.7 g of etoposide was combined with 1.7 g of PVA and the milling time was 14 days. The final particle size was 310 nm. The particles were stable in acid and plasma.

EXAMPLE 9

Example 8 was repeated except that the PVA was replaced by Pluronic F-108 (BASF). The final particle size was 312 nm. The particles were stable in acid and plasma.

EXAMPLE 10

Etoposide (2%) was milled in sterile water for 7 days. A 1:1 mixture of the milled slurry was prepared with 2% Pluronic F127 solution. The mixture was vortexed before measuring particle size. The final size was 277 nm. The slurry was stable in simulated gastric fluid, PBS (pH 7.4) and rat plasma.

EFFICACY STUDIES

Nanoetoposide formulations were evaluated in two separate efficacy studies in pancreatic ductal adenocarcinoma #03 (PANC #03). Control C was a 2% nonaqueous etoposide solution prepared using the formula described on pages 741–743 of the 46th Edition of the Physicians' Desk Reference. As described above, antitumor activity was assessed by monitoring tumor weight from experimental and control animals. These studies demonstrate that the etoposide compositions of this invention provide a means to deliver high doses of the drug without evidence of severe toxic reaction.

1. Efficacy Studies for Nanoetoposide in PANC #03 Murine Tumor Model

Example	Route	Dose mg/kg	% Wt Loss	Drug Deaths	T/C %
Control C	IV	120	−24.0	0/5	4.0
	IV	75	−4.0	0/5	20.0
7	IV	160	−12.0	0/5	18.0
	IV	100	0.0	0/5	32.0
	IV	62	2.0	0/5	42.0
8	IV	160	−12.0	0/5	26.0
	IV	100	+2.0	0/5	35.0
	IV	62	+4.0	0/5	35.0
9	IV	170	−18.5	1/5	16
	IV	85	−2.0	0/5	35
	IV	43	+2.5	0/5	41

EXAMPLES 11–16 Nanoparticulate Taxol

EXAMPLE 11

Approximately 18 mL of precleaned zirconium oxide media (1 mm) was added to a 30 mL amber jar. To it was added 240 mg of taxol (Sigma Chemicals) and 180 mg of Tween 20. Finally, 12 mL of water for injection was added to the jar, it was sealed and mounted on a roller mill for 11 days. The final particle size was 327 nm. The formulation was stable when exposed to PBS (pH 7.4) and rat plasma.

EXAMPLE 12

Example 11 was repeated except that the Tween 20 was replaced with PVA (MW 30 to 70 k). The final particle size was 365 nm.

The above samples were evaluated in efficacy studies in mice bearing early stage mammary adenocarcinoma #16/C. The antitumor activity was assessed by comparing tumor weights of taxol treated animals with the tumor weights of untreated animals. The toxicity was assessed by dose ranging studies with death and weight loss as end points. All samples were injected IV.

Example	Dose mg/kg	% Wt Loss	Deaths	Median Tumor on Day 11	T/C %
Control D	—	+6.1	—	1539 mg	—
Example 11	108.5	−1.5	0/5	1528	13
Example 12	108.5	−3.0	0/5	201	99

A control sample of taxol (NCI) was not available. However, a single dose of taxol formulated in Cremophore EL causes immediate deaths at 25 mg/kg total dose. However, taxol formulated in compositions of this invention could be injected at a dose of 88 mg/kg with no apparent adverse effects.

A taxol suspension prepared in a manner similar to Example 11 was treated separately with several surface modifiers. After addition of the new surface modifier the mixture was vortexed and evaluated for particle size and fluid stability. All the suspensions contained 1% taxol and 0.75% Tween 20. The results are as follows.

Example/ Surface Modifier	Concentration %	Size (nm)	Fluid Stability PBS	Fluid Stability Rat Plasma
13 CTAC	0.25	364	OK	Flocculation
14 AOT	0.25	322	SA*	SA
15 F68	0.5	297	SA/OK	SA/OK

-continued

Example/ Surface Modifier	Concentration %	Size (nm)	Fluid Stability PBS	Fluid Stability Rat Plasma
16 T908	0.5	313	SA/OK	SA/OK

*SA = Slight Aggregation

EXAMPLES 17–18 Nanoparticulate WIN 59075

Approximately 60 mL of precleaned zirconium oxide (1 mm) media was transferred into a 4 oz amber jar. It was followed by addition of 1.5 g of WIN 59075 and 28.5 mL of water for injection. The jars were sealed, loaded onto a roller mill and cascaded at 95 RPM for 48 hrs. PCS analysis determined the particle size to be 322 nm, however, the presence of larger particles was suggested. Milling was continued for 5 additional days.

Studies were conducted by mixing 0.5 mL of the WIN 59075 slurry prepared above with 0.5 mL of 6% surface modifier solutions. The final concentration of the drug was 2.5% and that of the surface modifiers was 3%. The surface modifier stabilized nanosuspensions of WIN 59075 were then treated with either PBS (pH 7.0) or 0.1N HCl (pH 1). Optical microscopic observations were made to determine fluid stability. The results are as follows;

Example	Surface Modifier	Stability pH 1	Stability pH 7	Human Plasma
17	PVP (12K) (BASF)	Fine	Fine	Fine
18	Gum Acacia (Aldrich)	—	SA/OK	SA/OK

It was concluded that stable nanoparticles of WIN 59075 could be prepared.

EXAMPLES 19–22 Nanoparticulate SR 4889

7.5 mL of precleaned zirconium oxide media (1 mm) was transferred into a 15 mL amber jar along with 18.75 mg of SR 4889 and 3.75 mL of water. After 11 days of milling the nanosuspension was separated from the media. To each of the 100 μL aliquots of the suspension, 100 μL of a surfactant solution (2%) was added giving a final concentration of 0.25% drug and 1% surfactant. The mixture was vortexed and analyzed for particle size. Fluid stability was assessed microscopically by mixing 10 μL of the suspension with 90 μL of rat plasma. The results are as follows:

Example	Surface Modifier	Particle Size (nm)	Fluid Stabilty Rat Plasma
19	PVP (12K)	134	Fine
20	Gum Acacia	344	SA/OK
21	Tween 80	128	Fine
22	T908	130	Aggregates

EXAMPLE 23 Nanoparticulate Retinoic Acid

30 mL of precleaned zirconium oxide media was transferred to a 60 mL amber jar. To it was added 1 g of transretinoic acid (Sigma), 470 mg of tyloxapol and 15 mL of water. The mixture was milled on a roller mill for 15 days. The final particle size was 140 nm. The nanosuspension was stable when exposed to either rat plasma or simulated gastric fluid.

The invention has been described in detail with particular reference to certain preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

What is claimed:

1. Particles consisting essentially of 99.9% by weight of a crystalline medicament useful in treating cancer susceptible to treatment with said medicament, said medicament having a solubility in water of less than 10 mg/ml, and having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1–90% by weight and sufficient to maintain an average effective particle size of less than 1000 nm, wherein said medicament is selected from the group consisting of alkylating agents selected from the group consisting of alkylating agents having a bis-(2-chloroethyl)-amine group, alkylating agents having a substituted aziridine group, alkyl sulfonates, and N-alkyl-N-nitrosoureas; antimetabolites; natural products selected from the group consisting of vinca alkaloids, epipophylotoxins, adriamycine, daunomycine, doctinomycine, daunorubicin, doxorubicin, mithramycin, bleomycin, mitomycin, enzymes, biological response modifiers, camptothecin, taxol and retinoids; hormones and antagonists: radiosensitizers; platinum coordination complexes; anthracenediones; and adrenocortical suppressants.

2. The particles of claim **1** having an average effective particles size of less than 400 nm.

3. The particles of claim **1** having an average effective particle size of less than 300 nm.

4. The particles of claim **1** wherein said surface modifier is present in an amount of 1 to 75% by weight.

5. The particles of claim **1** wherein said anticancer agent is selected from the group consisting of piposulfan, piposulfam, camptothecin, etoposide, taxol, 1,2,4-benzotriazin-3-amine 1,4-dioxide, 1,2,4-benzotriazin-7-amine 1,4-dioxide and retinoic acid.

6. The particles of claim **1** wherein said surface modifier is selected from the group consisting of polyvinyl alcohol, a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine, gum acacia, a block copolymer of ethylene oxide and propylene oxide, a polyoxyethylene sorbitan fatty acid ester, and a sorbitan ester of a fatty acid.

7. The particles of claim **1** wherein said anticancer agent is taxol and said surface modifier comprises a polyoxyethylene sorbitan fatty acid ester.

8. An anticancer composition comprising the particles of claim **1**.

9. A method of treating a mammal comprising administering to the mammal an effective amount of the anticancer composition of claim **8**.

10. In a method of treating a mammal comprising administering to the mammal an effective amount of an anticancer agent, the improvement wherein the efficacy of said anticancer agent is increased by administering said anticancer agent in the form of the particles of claim **1**.

11. In a method of treating a mammal comprising administering to the mammal an effective amount of an anticancer agent, the improvement wherein the toxicity of said anticancer agent is reduced by administering said anticancer agent in the form of the particles of claim **1**.

12. The particles of claim **1** wherein said surface modifier is a surfactant.

13. The particles of claim 1 wherein said surface modifier is a nonionic surfactant.

14. The particles of claim 1 wherein said surface modifier is an anionic surfactant.

15. The particles of claim 1 wherein said surface modifier is selected from the group consisting of gelatin, casein, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinyipyrrolidone, poloxomers, tyloxapol, poloxamines, dextran, a dioctyl ester of sodium sulfosuccinic acid, sodium lauryl sulfate, an alkyl aryl polyether sulfonate, a mixture of sucrose stearate and sucrose distearate, hexyldecyl trimethyl ammonium chloride, bovine serum albumin and $C_{18}H_{37}CH_2(CON(CH_3)CH_2(CHOH)_4CH_2OH)_2$.

16. The particles of claim 1 wherein said surface modifier is present in an amount of 10 to 60% by weight based on the total weight of the dry particle.

17. The particles of claim 1 wherein said surface modifier is present in an amount of 10 to 30% by weight based on the total weight of the dry particle.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

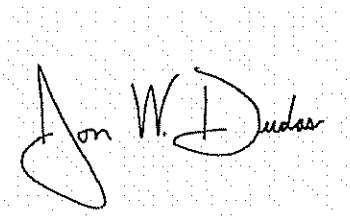
PATENT NO. : 5,399,363 Page 1 of 1
DATED : March 21, 1995
INVENTOR(S) : Gary G. Liversidge, Elaine Liversidge and Pramod P. Sarpotdar

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 14,
Line 7, delete "99.9%" and insert -- 99.9-10% --.

Signed and Sealed this

Twenty-fifth Day of April, 2006



JON W DUDAS
Director of the United States Patent and Trademark Office

EXHIBIT B

US005834025A

United States Patent [19]

de Garavilla et al.

[11] Patent Number: 5,834,025

[45] Date of Patent: Nov. 10, 1998

[54] **REDUCTION OF INTRAVENOUSLY ADMINISTERED NANOPARTICULATE-FORMULATION-INDUCED ADVERSE PHYSIOLOGICAL REACTIONS**

[75] Inventors: **Lawrence de Garavilla**, Downingtown; **Elaine M. Liversidge**; **Gary G. Liversidge**, both of West Chester, all of Pa.

[73] Assignee: **Nanosystems L.L.C.**, King of Prussia, Pa.

[21] Appl. No.: **696,754**

[22] Filed: **Aug. 14, 1996**

Related U.S. Application Data

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[51] **Int. Cl.**[6] **A61K 9/50**; B32B 5/16; B01J 13/02

[52] **U.S. Cl.** **424/501**; 424/502; 428/402.21; 264/4.3

[58] **Field of Search** 424/501, 502; 428/402.21; 264/4.3

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,565,188 10/1996 Wong et al. 424/499

Primary Examiner—Carlos Azpuru
Attorney, Agent, or Firm—Foley & Lardner

[57] **ABSTRACT**

Disclosed are methods of intravenous administration of nanoparticulate drug formulations to a mammal to avoid adverse hemodynamic effects: by reducing the rate and concentration of the nanoparticles in the formulations; or by pre-treating the subject with histamine; or by pretreating the subject with a desensitizing amount of the nanoparticulate drug formulations.

20 Claims, No Drawings

REDUCTION OF INTRAVENOUSLY ADMINISTERED NANOPARTICULATE-FORMULATION-INDUCED ADVERSE PHYSIOLOGICAL REACTIONS

BACKGROUND OF THE INVENTION

This application claims the benefit under 35 USC 119(*e*) of Provisional Application Number 60/004,488 filed Sep. 29, 1995

Field of the Invention

The present invention is directed to nanoparticulate, liposome, emulsion and polymeric colloidal drug formulations for intravenous administration, the delivery of which to a mammal, reduces/eliminates the adverse physiological effects. More particularly, the invention relates to a method of intravenous nanoparticulate, liposome, emulsion and polymeric colloidal drug formulations to a mammal, wherein the rate of infusion and concentration of the drug is controlled.

Reported Developments

Nanoparticles are well know in the prior art, having been described, for example, in U.S. Pat. No. 5,145,684. These particles consist of a crystalline drug substance having a surface modifier adsorbed on the surface of the particles such that the average particle size is less than about 400 mm. Low solubility of solid drugs prompted the pharmaceutical industry to create nanoparticles of such drugs which then can be administered systematically to provide bioavailability. Drug substances disclosed which can be made into nanoparticles include a variety of known classes of drugs.

In order to provide nanometer-size particles, the drug substance is comminuted in the presence of a surface active agent or, alternatively, the surface active agent is allowed to be adsorbed to the nanoparticulate drug substance after the process of comminution. The surface active agent prevents flocculation or congregation of the nanoparticles. The achievements of nanoparticulate technology in the pharmaceutical industry affords the opportunity to prepare parenteral formulations of water-insoluble or poorly water-soluble drugs. These formulations by their nature are suspensions rather than solutions since the particles are dispersed/suspended in a pharmaceutically acceptable vehicle. Liposomes, emulsions and colloids when used as carriers for an active drug are also suspensions rather than solutions.

We have observed that intravascular adminstration of a suspensions to dogs causes significant cardiovascular dysfunction, such as reduction in arterial blood pressure and cardiac function, including heart rate cardiac output and ventricular contractility.

Other investigators have reported similar findings, for example: Slack et al., Acute Hemadynamic Effects and Blood Pool Kinetics of Polystyrene Microspheres Following Intravenous Administration, J. Pharm. Sci., 660, 1981; Faithfull et al., Cardiorespiratory Consequences of Fluro-carbon Reactions in Dogs, Bio. Art. Organs, 1, 1988; and Lorenz et al., Histamine Release in Dogs by Cremophore EL and its Derivatives, Agents and Actions, 63, 1977.

It has now been discovered that hemodynamic effects of suspensions can be eliminated or at least substantially reduced by controlling the rate of infusion and/or the concentration of the active drug in the suspensions.

The present invention will be described particularly in reference to nanoparticulate crystalline drug formulations, however, the invention encompasses the use of other carriers for active drugs that do not form a solution but rather a suspension or dispersion having nanoparticulates in the range of less than 1000 nm.

SUMMARY OF THE INVENTION

This invention provides a method of administering a nanoparticulate composition to a mammal such as a dog or other sensitive species without eliciting adverse hemodynamic effects comprising:

intravenously administering to said dog an effective amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min comprising particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline organic drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a surface modifier adsorbed on the surface thereof in an amount of 0.1–99.9% by weight and sufficient to maintain an effective average particle size of from about 50 to about 1000 nm, and a pharmaceutically acceptable carrier therefor.

In another embodiment, the present invention provides a method of administering a nanoparticulate composition to a mammal such as a dog or other sensitive species without eliciting adverse hemodynamic effects comprising:

intravenously administering to said dog an antihistamine including H1, H2 and H3 receptor antagonists in the amount of form about 5 to about 10 mg/kg of body weight, such as diphenhydramine, cimetidine or thioperamide or a combination thereof followed by intravenously administering to said dog an effective amount of a nanoparticulate drug composition comprising particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a surface modifier adsorbed on the surface thereof in an amount of 0.1–99.0% by weight and sufficient to maintain an effective average particle size of less than 1000 nm, and preferably less than about 400 nm, and a pharmaceutically acceptable carrier thereof.

In still another embodiment the present invention provides a method of administering a nanoparticulate composition to a mammal, such as a dog or other sensitive species without eliciting adverse hemodynamic effects comprising: intravenously administering to said mammal a desensitizing amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min, followed by intravenous administration of an effective amount of said nanoparticulate composition comprising particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline organic drug substance having a solubility in water of less than 10 mg/ml, said drug substance having a surface modifier adsorbed on the surface thereof in an amount sufficient to maintain an effective average particle size of from 50 to 1000 nm, and a pharamceutically acceptable carrier therefor.

In a liposome- or colloidal-drug delivery system, the bioactive drug is entrapped in the liposome or colloidal particles in an amount of from about 0.1 to about 99.9 to about 99.1 to about 0.1% by weight liposome or colloidal particles, such particles having a particle size of less than 1000 mm, and then administered to the patient to be treated.

As used herein, the term "desensitizing amount" means a dose low enough so as not to elicit a response but still acutely precondition the mammal so that a subsequent injection of a full dose will now be without effect.

Also, as used herein, mammal preferably means a dog and other sensitive species including human species.

DETAILED DESCRIPTION OF THE INVENTION

Nanoparticulate formulations cause a significant transient decrease in arterial blood pressure immediately following intravenous injection in anesthetized dogs. Extensive studies were conducted to evaluate the acute hemodynamic effects of intravenously administered nanoparticles. To eliminate effects which may be caused by an active drug, inert polystyrene nanoparticles were used in the studies. Parameters were monitored throughout the studies including mean arterial pressure, heart rate, mean pulmonary arterial pressure, left ventricular systolic pressure, temperature, blood chemistry and hematology. In order to distinguish and separate the effects arising from components of nanoparticulate formulations, the effects of intravenous injections of: inert polystyrene nanoparticles, surfactant-coated polystyrene nanoparticles and surfactant alone were used in separate groups of dogs in the studies.

STUDY DESIGN/METHODS

A. Animals

Mongrel dogs of either sex weighing between 13 and 16 kg were used in these studies. Dogs were acquired form Butler Farms (Clyde, N.Y.) and allowed at least a two week acclimation period following shipment and prior to conducting these studies.

B. Anesthesia and Surgical Preparation

Dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Following surgical preparation, the dogs were placed on an infusion of sodium pentobarbital (4 mg/kg/hr) to maintain an adequate, constant level of anesthesia throughout the experimental protocol. All anesthetic injections were administered via a 20 gauge percutaneous venicatheter placed in the right cephalic vein.

Following induction of anesthesia, dogs were placed in dorsal recumbency and intubated with a 7 French endotracheal tube and allowed to breathe room air. (Core temperature, as measured using a thermistor probe inserted 6 cm into the colon, was maintained between 37.5° and 38.5° C. using a thermostatically controlled heating pad (Harvard Homeothermic Heating Blanket, Harvard Instruments, Natick, Mass.).

The right carotid artery, right femoral artery, and the right and left femoral veins were surgically isolated via percutaneous incisions and blunt dissection. A high fidelity, solid state, dual sensor pressure transducer catheter (Model SPC 784A, Millar Instruments, Houston, Tex.) was advanced retrograde from the carotid artery and placed into the left ventricular chamber of the heart. The distal pressure sensor, positioned in the left ventricle, was used to measure intraventricular pressure and the proximal sensor, positioned at the root of the aorta, measured intra-aortic pressure. The pulsatile left ventricular pressure (LVP) signal was electronically differentiated (dP/dt) to acquire an estimate of cardiac contractility (LV dP/dt). Heart rate (HR) was also derived from the pulsatile LVP signal. Mean arterial blood pressure (MAP) was derived form the pulsatile signal of intraaortic pressure. Another fluid-filled catheter was placed in the right femoral artery for acquisition of arterial blood samples for blood gas measurements. Arterial blood pH, PCO_2 and $_pO_2$ were measured on freshly drawn samples using an automated blood gas analyzer (Model ABL30, Radiometer Corp., Westlake, Ohio). Platinum pin electrodes were inserted subcutaneously to monitor lead II electrocardiogram. All particles were injected in the left cephalic vein via a 20 gauge percutaneous venicatheter.

Following surgical preparation and instrumentation, the dogs were allowed a 30 minute acclimation period prior to initiation of the test.

C. Data Acquisition and Analysis

The following variables were continuously recorded on a pc-based automated data acquisition system (Modular Instruments, Inc., Malvern, Pa.):

Mean arterial blood pressure (MAP; mmHg)

Heart rate (HR; min^{-1})

Mean pulmonary arterial blood pressure (PAP; mmHg)

Left ventricular systolic blood pressure (LVP_{sys}; mmHg)

Maximum left ventricular dP/dt (+LV dP/dt mmHg sec^{-1})

Maximum left ventricular dP/dt (−LV dP/dt mmHg sec^{-1})

The following variable were mathematically derived from the above variables and cardiac output (CO; L min^{-1}):

Systemic arterial vascular resistance (SVR; calculated as MAP/CO; mmHg L^{-} min)

Pulmonary vascular resistance (PVR; calculated as PAP/CO; mmHg L^{-1} min)

Stroke volume (SV; calculated as CO/HR.1000; cc)

Data were down-loaded onto a PC-based statistical package CRUNCH (Crunch Software Corporation, Oakland, Calif.). An analysis of variance followed by a Dunnett's comparison was used to test the effect of dose within each group. Between group effects (WIN 8883 vs. vehicle) were analyzed using a t-test. In all cases, data are expressed as mean±s.e.

D. Drugs and Solutions

Polystyrene Particles (Surfactant Coated)

Polystyrene particles having a diameter of 200 nm were obtained form Polysciences, Inc.

1) The Vehicle (Placebo)

A 10% solution of F108 was made by weighing out 1 g of F108 in a 50 ml plastic beaker and then adding 8 ml of water (Sterile water for injection—SWFI) to the surfactant. The beaker was placed in the refrigerator until the surfactant had gone into solution. SWIF was added to bring the final volume to 10 mls which was then filtered with a 0.1 μm syringe filter before use.

2) 5% Polystyrene Particles (PSP)+5% F108

To prepare 20 mls of 5% PSP+5% F108, 10 mls of the 200 nm particles were added to 10 mls of 10% F108 in a 50 ml centrifuge tube. The tube was placed on a mutator and the suspension was allowed to mix for 1 hour. Solutions were also prepared with 100 and 50 nm particle size polystyrene particles.

3) 5% WIN 8883+5% P108

The suspension of 5% WIN 8883+5% F108 was prepared analogously to the 5% Polystyrene+5% F108 suspension shown in #2.

4) Supernatant of 5% WIN 8883

Supernatant of WIN 8883 was prepared by centrifugation of a sample of WIN 8883 at 20,000 g for 1 hour, followed by recentrifugation of the decanted supernatant 5% WIN 8883. No WIN 8883 was found in the decanted solution.

5) Supernatant of 5% Polystyrene+5% F108

The supernatant was prepared analogously to that shown in #4. No polystyrene was found in the supernatant.

The preparations of #1, #2, #3, #4 and #5 were administered to groups of dogs subcutaneously and intravenously at various dose levels described below. Baseline hemodynamics were recorded for a 10 minute period just prior to administration of the preparations. Following administration of the preparation each dog was monitored 60 minutes.

E. Results

Results of testing are shown in the following tables.

TABLE 1

Effect of the vehicle 5% F108 and a particulate-free supernatant on the hemodynamic and hematological response in dogs when infused at a rate of 5 ml/min and a dose of 0.1 ml/kg. Values listed as means ± S.E.

Treatment	N	% Change from Baseline MAP
#5% F108	4	−1 ± 1
#5 Supernatant	3	−1 ± 1

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

TABLE 2

Effect of uncoated and F108 (5%) coated 200 nM diameter polystyrene nanospheres, 5% suspension, infused at a rate of 1 ml/min and a dose of 0.1 ml/kg on the hemodynamic and hematological response in dogs Values listed as means ± S.E.

Treatment	N	% Change from Baseline MAP
#4 Uncoated	3	−1 ± 1
#3 Coated	4	−50 ± 7

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

TABLE 3

Effect of rate of infusion of 200 nM diameter polystyrene nanospheres on the hemodynamic and hematological response in dogs. Each formulation was prepared as a 1% (w:v) suspension in a solution of 5% F108 and administered intravenously at a dose of 0.1 ml/kg. Values listed as means ± S.E.

Rate (ml/min)	N	% Change from Baseline MAP
5	3	−39 ± 15
1	4	−26 ± 5
0.5	3	−5 ± 1

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

TABLE 4

Effect of rate of infusion of 200 nM polystyrene nanospheres on the hemodynamic and hematological response in dogs Each formulation was prepared as a 5% (w:v) suspension in a solution of 5% F108 and adminstered intravenously at a dose of 0.1 ml/kg. Values listed as means ± S.E.

Rate (ml/min)	N	% Change from Baseline MAP
1	4	−50 ± 7
0.1	3	−1 ± 1

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

TABLE 5

Effect of pretreatment with antihistamines on the hemodynamic and hematological response in dogs following ontravenous administration of a 1% (w:v) suspension of polystyrene nanospheres, 200 nM in diameter, in a 5% solution of F108 at a dose of 0.1 ml/kg and a rate of 5 ml/min. Values listed as means ± S.E.

Antihistamine	N	% Change from Baseline MAP
None	3	−39 ± 15
Diphenhydramine 10 mg/kg	4	−7 ± 1
Cimetidine 5 mg/kg		
Diaphenhydramine 10/mg/kg	3	−4 ± 2
Cimetifine 5 mg/kg		
Thioperamide 5 mg/kg		

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

TABLE 6

Effect of particle size on the hemodynamic and hematological response in dogs Each formulation was prepared as a 1% (w:v) suspension in a 5% solution of F108 and administered intravenously at a dose of 0.1 ml/kg and a rate of 5 ml/min Values listed as means ± S.E.

Diameter (nM)	N	% Change from Baseline MAP
200	3	−39 ± 15
100	4	−26 ± 14
50	3	−8 ± 5

wherein:
N = no. of dogs
MAP = Mean Arterial Blood Pressure

The results are summarized in Table 7

TABLE 7

% Solids (w/v)	Infusion Rate (ml/min)	Solids Dose Rate (mg/ml/min)	Response Y or N
5 (50 mg/ml)	1	50	Y
1 (10 mg/ml)	10	100	Y
1 (10 mg/ml)	5	50	Y
1 (10 mg/ml)	1	10	Y
5 (50 mg/ml)	0.1	5	N
10 ng/ml	0.5	5	N

The studies indicate that there is a critical set of parameters which control hemodynamic effects in dogs To avoid overt deleterious hemodynamic effects the following parameters should be controlled:

1) The vehicle-surfactant administered alone does not initiate a hemodynamic response.

2) "uncoated" polystyrene nanoparticles administered at a dose of 0.1 ml/kg of body weight and a rate of 1 ml/min does not induce hemodynamic effect. However, "uncoated" nanoparticle drugs cannot be administered safely, since they coagulate/flocculate causing deleterious side effects Accordingly, active drugs must be surfactant coated to circumvent coagulation/flocculation of the nanoparticles

3) Hemodynamic effect is associated with the surfactant-particle combination While the inventors would not want to be limited to a theory of mechanism in the practice of their invention, it appears that the particles act as carrier for the surfactant, delivering it to its site of action in the blood stream. Theoretically, the uncoated particle also accesses the site of action but cannot initiate the response in the absence

of the surfactant. Conversely, in the absence of particles, the surfactant also cannot access the site of action and does not initiate the response.

4) At a concentration of 1% solids, at 0.1 ml/kg and at infusion rate of 10 ml/min hemodynamic effects occur. At 5 ml/min delivery of the hemodynamic effect was about half of that of the 10 ml/min infusion.

5) At particle size of less than about 100 nm no hemodynamic effect occurs, while hemodynamic effect increases from 100 nm to 200 nm particle size and above 200 nm particle size.

6) Pretreatment with antihistamines to avoid hemodynamic effects. Plasma histamine levels appear to be elevated during the response. Thus the ability to block the effects of histamine at its receptor site can block the response or at least mitigate it.

Based on these and other studies the present invention provides methods which can be utilized to deliver effective amounts of drugs without eliciting adverse hemodynamic effects in dogs. The drug formulations and their methods of preparations will now be described.

Drugs

The particles comprise a therapeutic or diagnostic agent. (therapeutic agents are sometimes referred to as drugs or pharmaceuticals. The diagnostic agent referred to is typically a contrast agent such as an x-ray contrast agent but can also be other diagnostic materials.) The therapeutic or diagnostic agent exists as a discrete, crystalline phase. The crystalline phase differs from a non-crystalline or amorphous phase which results from precipitation techniques, such as described in EPO 275,796.

The invention can be practiced with a wide variety of therapeutic or diagnostic agents. The therapeutic or diagnostic agent preferably is present in an essentially pure form. The therapeutic or diagnostic agent must be poorly soluble and dispersible in at least one liquid medium. By "poorly soluble" it is meant that the therapeutic or diagnostic agent has a solubility in the liquid dispersion medium of less than about 10 mg/ml, and preferably of less than about 1 mg/ml. A preferred liquid dispersion medium is water. However, the invention can be practiced with other liquid media in which a therapeutic or diagnostic agent is poorly soluble and dispersible including, for example, aqueous salt solutions, safflower oil and solvents such as ethanol, t-butanol, hexane and glycol. The pH of the aqueous dispersion media can be adjusted by techniques known in the art.

Suitable therapeutic or diagnostic agents can be selected from a variety of known classes of therapeutic or diagnostic agents including, for example, anti-inflammatory agents, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives (hypnotics and neuroleptics), beta-adrenoceptor blocking agents, cardiac inotropic agents, contrast media, corticosteroids, diagnostic agents, diagnostic imaging agents, dopaminergics (antiparkinsonian agents), haemostatics, immuniological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators and xanthines. A description of these classes of therapeutic agents and diagnostic agents and a listing of species within each class can be found in Martindale, *The Extra Pharmacopoeia*, Twenty-ninth Edition, The Pharmaceutical Press, London, 1989. The therapeutic or diagnostic agents are commercially available and/or can be prepared by techniques known in the art.

Preferred diagnostic agents include the x-ray imaging agent WIN-8883 (ethyl 3,5-diacetamido-2,4, 6triiodobenzoate) also known as the ethyl ester of diatrazoic acid (EEDA), WIN 67722, i.e., (6-ethoxy-6-oxohexyl-3,5-bis(acetamido)- 2,4,6-triiodobenzoate; ethyl-2-(3,5-bis (acetamido)-2,4,6-triiodobenzoyloxy)butyrate (WIN 16318); ethyl diatrizoxyacetate (WIN 12901); ethyl 2-(3, 5bis(acetamido)-2,4,6-triiodobenzoyloxy)propionate (WIN 16923); N-ethyl 2-(3,5-bis(acetamido)-2,4, 6triiodobenzoyloxy acetamide (WIN 65312); isopropyl 2(3, 5-bis(acetamido)-2,4,6-triiodobenzoyloxy) acetamide (WIN 12855); diethyl 2-(3,5-bis(acetamido)-2,4, 6triiodobenzoyloxy malonate (WIN 67721); ethyl 2-(3,5bis (acetamido)-2,4,6-triiodobenzoyloxy) phenylacetate (WIN 67585); propanedioic acid, [[3,5-bis(acetylamino)2,4,5-triodobenzoyl]oxy]-,bis(1-methyl)ester (WIN 68165); and benzoic acid, 3,5-bis(acetylamino) 2,4,6triodo-, 4-(ethyl-3-ethoxy-2-butenoate) ester (WIN 68209). Suitable diagnostic agents are also disclosed in U.S. Pat. No. 5,260,478; U.S. Pat. No. 5,264,610; U.S. Pat. No. 5,322,679 and U.S. Pat. No. 5,300,739.

Preferred contrast agents include those which are expected to disintegrate relatively rapidly under physiological conditions, thus minimizing any particle associated inflammatory response.

Surface Modifiers

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and ionic surfactants.

Representative examples of surface modifiers include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens™, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxy propylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone (PVP). Most of these surface modifiers are known pharmaceutical excipients and are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986.

Particularly preferred surface modifiers include polyvinylpyrrolidone, tyloxapol, poloxamers such as Pluronics™ F68 and F108, which are block copolymers of ethylene oxide and propylene oxide, and polyxamines such as Tetronics™ 908 (also known as Poloxamine™ 908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to

ethylenediamine, available from BASF, dextran, lecithin, dialkylesters of sodium sulfosuccinic acid, such as Aerosol OTs™, which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanimid, Duponols™ P, which is a sodium lauryl sulfate, available from DuPont, Tritons™ X-200, which is an alkyl aryl polyether sulfonate, available from Rohn and Haas, Tween™ 20 and Tweens™ 80, which are polyoxyethylene sorbitan fatty acid esters, available from ICI Specialty Chemicals; Carbowaxs™ 3550 and 934, which are polyethylene glycols available from Union Carbide; Crodestas™ F-110, which is a mixture of sucrose stearate and sucrose distearate, available from Croda Inc., Crodestas™ SL-40, which is available from Croda, Inc., and SA9OHCO, which is $C_{18}H_{37}CH_2(CON(CH_3)CH_2(CHOH)_4(CH_2OH)_2$. Surface modifiers which have been found to be particularly useful include Tetronics™ 908, the Tweenss™, Pluronics™ F-68 and polyvinylpyrrolidone. Other useful surface modifiers include:

decanoyl-N-methylglucamide;

n-decyl β-D-glucopyranoside;

n-decyl β-D-maltopyranoside;

n-dodecyl β-D-glucopyranoside;

n-dodecyl β-D-maltoside;

heptanoyl-N-methylglucamide;

n-heptyl-β-D-glucopyranoside;

n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside;

nonanoyl-N-methylglucamide;

n-noyl β-D-glucopyranoside;

octanoyl-N-methylglucamide;

n-octyl-β-D-glucopyranoside;

octyl β-D-thioglucopyranoside; and the like.

Another useful surface modifier is tyloxapol (a nonionic liquid polymer of the alkyl aryl polyether alcohol type; also known as superinone or triton). This surface modifier is commercially available and/or can be prepared by techniques known in the art.

Another preferred surface modifier is p-isononylphenoxypoly(glycidol) also known as Olin-10G™ or Surfactant 10-G, is commercially available as 10G™ from Olin Chemicals, Stamford, Conn.

Non-Ionic Surface Modifiers

Preferred surface modifiers can be selected from known non-ionic surfactants, including the poloxamines such as Tetronic™908 (also known as Poloxanmine™908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine, available from BASF, or Tetronic™ 1508 (T-1508), or a polymer of the alkyl aryl polyether alcohol type, such as tyloxapol.

The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two-or more surface modifiers can be used in combination.

Auxiliary Surface Modifiers

Particularly preferred auxiliary surface modifiers are those which impart resistance to particle aggregation during sterilization and include dioctylsulfosuccinate (DOSS), polyethylene glycol, glycerol, sodium dodecyl sulfate, dodecyl trimethyl ammonium bromide and a charged phospholipid such as dimyristoyl phophatidyl glycerol. The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

Block Copolymer Surface Modifiers

One preferred surface modifier is a block copolymer linked to at least one anionic group. The polymers contain at least one, and preferably two, three, four or more anionic groups per molecule.

Preferred anionic groups include sulfate, sulfonate, phosphonate, phosphate and carboxylate groups. The anionic groups are covalently attached to the nonionic block copolymer. The nonionic sulfated polymeric surfactant has a molecular weight of 1,000–50,000, preferably 2,000–40,000 and more preferably 3,000–30,000. In preferred embodiments, the polymer comprises at least about 50%, and more preferably, at least about 60% by weight of hydrophilic units, e.g., alkylene oxide units. The reason for this is that the presence of a major weight proportion of hydrophilic units confers aqueous solubility to the polymer.

A preferred class of block copolymers useful as surface modifiers herein includes sulfated block copolymers of ethylene oxide and propylene oxide. These block copolymers in an unsulfated form are commercially available as Pluronics™. Specific examples of the unsulfated block copolymers include F68, F108 and F127.

Another preferred class of block copolymers useful herein include tetrafunctional block copolymers derived from sequential addition of ethylene oxide and propylene oxide to ethylene diamine. These polymers, in an unsulfated form, are commercially available as Tetronics™.

Another preferred class of surface modifiers contain at least one polyethylene oxide (PEO) block as the hydrophilic portion of the molecule and at least one polybutylene oxide (PBO) block as the hydrophobic portion. Particularly preferred surface modifiers of this class are diblock, triblock, and higher block copolymers of ethylene oxide and butylene oxide, such as are represented, for example, by the following structural formula:

$$-(PEO)_n-(PBO)_n-(PEO)_n-(PBO)_n-(PEO)_n- \text{ and } -(PEO)_n-(PBO)_n-(PEO)_n-(PBO)_n-$$

The block copolymers useful herein are known compounds and/or can be readily prepared by techniques well known in the art.

Highly preferred surface modifiers include triblock copolymers of the $-(PEO)_n-(PBO)_n-(PEO)_n-$ having molecular weights of 3800 and 5000 which are commercially available from Dow Chemical, Midland, Michigan, and are referred to as B20-3800 and B20-5000. These surface modifiers contain about 80% by weight PEO. In a preferred embodiment, the surface modifier is a triblock polymer having the structure:

$$R-Q\left(CH_2CH_2O\right)_x\left[CH_2CHO\right]_y\left(CH_2CH_2O\right)_z Q-R$$

(with C_2H_5 on the CH_2CHO unit)

Q is an anionic group

wherein R is H or a metal cation such as Na+, K+ and the like, x is 15-700, Y is 5-200 and z is 15-700.

Grinding

The described particles can be prepared in a method comprising the steps of dispersing a therapeutic or diagnostic agent in a liquid dispersion medium and applying mechanical means in the presence of grinding media to reduce the particle size of the therapeutic or diagnostic agent to an effective average particle size of less than about 1000

nm, and preferably of less than about 400 nm. The particles can be reduced in size in the presence of a surface modifier. Alternatively, the particles can be contacted with a surface modifier after attrition.

The therapeutic or diagnostic agent selected is obtained commercially and/or prepared by techniques known in the art in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse therapeutic or diagnostic agent selected be less than about 10 mm as determined by sieve analysis. If the coarse particle size of the therapeutic or diagnostic agent is greater than about 100 mm, then it is preferred that the particles of the therapeutic or diagnostic agent be reduced in size to less than 100 mm using a conventional milling method such as airjet or fragmentation milling.

The coarse therapeutic or diagnostic agent selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the therapeutic or diagnostic agent in the liquid medium can vary from about 0.1–60%, and preferably is from 5–30% (w/w). It is preferred, but not essential, that the surface modifier be present in the premix. The concentration of the surface modifier can vary from about 0.1 to about 90%, and preferably is 1–75%, more preferably 20–60%, by weight based on the total combined weight of the therapeutic or diagnostic agent and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise.

The premix can be used directly by subjecting it to mechanical means to reduce the average particle size in the dispersion to less than 1000 nm. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the therapeutic or diagnostic agent and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates-visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition. Alternatively, the therapeutic or diagnostic agnet and, optionally, the surface modifier, can be dispersed in the iquid medium using suitable agitiation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the particle size of the therapeutic or diagnostic agent conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, and media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, desired reduction in particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

Preparation Conditions

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills, processing times of up to five days or longer may be required. On the other hand, processing times of less than 1 day (residence times of one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a temperature which does not significantly degrade the therapeutic or diagnostic agent. Processing temperatures of less than about 30°–40° C. are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Control of the temperature, e.g., by jacketing or immersion of the milling chamber in ice water are contemplated. Processing pressures from about 1 psi (0.07 kg/cm2) up to about 50 psi (3.5 kg/cm2) are contemplated. Processing pressures from about 10 psi (0.7 kg/cm2) to about 20 psi 1.4 kg/cm2)

The surface modifier, if it was not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix above. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20–80 kHz for a time of about 1 to 120 seconds.

After attrition is completed, the grinding media is separated from the milled particulate product (in either a dry or liquid dispersion form) using conventional separation techniques, such as by filtration, sieving through a mesh screen, and the like.

Grinding Media

The grinding media for the particle size reduction step can be selected from rigid media preferably spherical or particulate in form having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical. We have found that zirconium oxide, such as 95% ZrO2 stabilized with magnesia, zirconium silicate, and glass grinding media provide particles having levels of contamination which are believed to be acceptable for the preparation of pharmaceutical compositions. However, other media, such as stainless steel, titania, alumina, and 95% ZrO2 stabilized with yttrium, are expected to be useful. Preferred media have a density greater than about 3 g/cm3.

Polymeric Grinding Media

The grinding media can comprise particles, preferably substantially spherical in shape, e.g., beads, consisting essentially of polymeric resin. Alternatively, the grinding media can comprise particles comprising a core having a coating of the polymeric resin adhered thereon.

In general, polymeric resins suitable for use herein are chemically and physically inert, substantially free of metals, solvent and monomers, and of sufficient hardness and friability to enable them to avoid being chipped or crushed during grinding. Suitable polymeric resins include crosslinked polystyrenes, such as polystyrene crosslinked with divinylbenzene, styrene copolymers, polycarbonates, polyacetals, such as Delrin™, vinyl chloride polymers and copolymers, polyurethanes, polyamides, poly (tetrafluoroethylenes), e.g., Teflon™, and other fluoropolymers, high density polyethylenes,

polypropylenes, cellulose ethers and esters such as cellulose acetate, polyhydroxymethacrylate, polyhydroxyethyl acrylate, silicone containing polymers such as polysiloxanes and the like. The polymer can be biodegradable. Exemplary biodegradable polymers include poly(lactides), poly (glycolide) copolymers of lactides and glycolide, polyanhydrides, poly(hydroxyethyl methacylate), poly (imino carbonates), poly(N-acylhydroxyproline)esters, poly (N-palmitoyl hydroxyproline) esters, ethylene-vinyl acetate copolymers, poly(orthoesters), poly(caprolactones), and poly(phosphazenes). In the case of biodegradable polymers, contamination from the media itself advantageously can metabolize in vivo into biologically acceptable products which can be eliminated from the body.

The polymeric resin can have a density from 0.8 to 3.0 g/cm3. Higher density resins are preferred inasmuch as it is believed that these provide more efficient particle size reduction.

The media can range in size from about 0.1 to 3 mm. For fine grinding, the particles preferably are from 0.2 to 2 mm, more preferably, 0.25 to 1 mm in size.

In a particularly preferred method, a therapeutic or diagnostic agent is prepared in the form of submicron particles by grinding the agent in the presence of a grinding media having a mean particle size of less than about 75 microns.

The core material of the grinding media preferably can be selected from materials known to be useful as grinding media when fabricated as spheres or particles. Suitable core materials include zirconium oxides (such as 95% zirconium oxide stabilized with magnesia or yttrium), zirconium silicate, glass, stainless steel, titania, alumina, ferrite and the like. Preferred core materials have a density greater than about 2.5 g/cm^3. The selection of high density core materials is believed to facilitate efficient particle size reduction.

Useful thicknesses of the polymer coating on the core are believed to range from about 1 to about 500 microns, although other thicknesses outside this range may be useful in some applications. The thickness of the polymer coating preferably is less than the diameter of the core.

The cores can be coated with the polymeric resin by techniques known in the art. Suitable techniques include spray coating, fluidized bed coating, and melt coating. Adhesion promoting or tie layers can optionally be provided to improve the adhesion between the core material and the resin coating. The adhesion of the polymer coating to the core material can be enhanced by treating the core material to adhesion promoting procedures, such as roughening of the core surface, corona discharge treatment, and the like.

Continuous Grinding

In a preferred grinding process, the particles are made continuously rather than in a batch mode. The continuous method comprises the steps of continuously introducing the therapeutic or diagnostic agent and rigid grinding media into a milling chamber, contacting the agent with the grinding media while in the chamber to reduce the particle size of the agent, continuously removing the agent and the grinding media from the milling chamber, and thereafter separating the agent from the grinding media.

The therapeutic or diagnostic agent and the grinding media are continuously removed from the milling chamber. Thereafter, the grinding media is separated from the milled particulate agent (in either a dry or liquid dispersion form) using conventional separation techniques, in a secondary process such as by simple filtration, sieving through a mesh filter or screen, and the like. Other separation techniques such as centrifugation may also be employed.

In a preferred embodiment, the agent and grinding media are recirculated through the milling chamber. Examples of suitable means to effect such recirculation include conventional pumps such as peristaltic pumps, diaphragm pumps, piston pumps, centrifugal pumps and other positive displacement pumps which do not use sufficiently close tolerances to damage the grinding media. Peristaltic pumps are generally preferred.

Another variation of the continuous process includes the use of mixed media sizes. For example, larger media may be employed in a conventional manner where such media is restricted to the milling chamber. Smaller grinding media may be continuously recirculated through the system and permitted to pass through the agitated bed of larger grinding media. In this embodiment, the smaller media is preferably between about 1 and 300 mm in mean particle size and the larger grinding media is between about 300 and 1000 mm in mean particle size.

Precipitation Method

Another method of forming the desired nanoparticle dispersion is by microprecipitation. This is a method of preparing stable dispersions of therapeutic and diagnostic agents in the presence of a surface modifying and colloid stability enhancing surface active agent free of trace of any toxic solvents or solubilized heavy metal inpurities by the following procedural steps:

1. Dissolving the therapeutic or diagnostic agent in aqueous base with stirring,

2. Adding above #1 formulation with stirring to a surface active surfactant (or surface modifiers) solution to form a clear solution, and

3. Neutralizing above formulation #2 with stirring with an appropriate acid solution. The procedure can be followed by:

4. Removal of formed salt by dialysis or diafiltration and

5. Concentration of dispersion by conventional means.

This microprecipitation process produces dispersion of therapeutic or diagnostic agents with Z-average particle diameter less than 400 nm (as measured by photon correlation spectroscopy) that are stable in particle size upon keeping under room temperature or refrigerated conditions. Such dispersions also demonstrate limited particle size growth upon autoclave-decontamination conditions used for standard blood-pool pharmaceutical agents.

Step 3 can be carried out in semicontinuous, continuous batch, or continuous methods at constant flow rates of the reacting components in computercontrolled reactors or in tubular reactors where reaction pH can be kept constant using pH-stat systems. Advantages of such modifications are that they provide cheaper manufacturing procedures for large-scale production of nanoparticulate dispersion systems.

Additional surface modifier may be added to the dispersion after precipitation. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonicationstep, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20–80 kHz for a time of about 1 to 120 seconds.

In a preferred embodiment, the above procedure is followed with step 4 which comprises removing the formed salts by diafiltration or dialysis. This is done in the case of dialysis by standard dialysis equipment and by diafiltration using standard diafiltration equipment known in the art. Preferably, the final step is concentration to a desired con-

centration of the agent dispersion This is done either by diafiltration or evaporation using standard equipment known in this art

An advantage of microprecipitation is that unlike milled dispersion, the final product is free of heavy metal contaminants arising from the milling media that must be removed due to their toxicity before product is formulated

A further advantage of the microprecipitation method is that unlike solvent precipitation, the final product is free of any trace of trace solvents that may be toxic and must be removed by expensive treatments prior to final product formulation

In another preferred embodiment of the microprecipitation process, a crystal growth modifier is used. A crystal growth modifier is defined as a compound that in the co-precipitation process incorporates into the crystal structure of the microprecipitated crystals of the pharmaceutical agent, thereby hindering growth or enlargement of the microcrystalline precipitate, by the so called Ostwald ripening process A crystal growth modifier (or a CGM) is a chemical that is at least 75% identical in chemical structure to the pharmaceuticl agent By "identical" is meant that the structures are identical atom for atom and their connectivity Structural identity is charactarized as having 75% of the chemical structure, on a molecular weight basis, identical to the therapeutic or diagnostic agent The remaining 25% of the structure may be absent or replaced by different chemical structure in the CGM The crystal growth modifier is dissolved in step #1 with the therapeutic or diagnostic agent

Particle Size

As used herein, particle size refers to a number average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. When photon correlation spectroscopy (PCS) is used as the method of particle sizing the average particle diameter is the Z-average particle diameter known to those skilled in the art. By "an effective average particle size of less than about 1000 nm" it is meant that at least 90% of the particles have a weight average particle size of less than about 1000 nm when measured by the above-noted techniques. In preferred embodiments, the effective average particle size is less than about 400 nm and more preferrably less than about 300 nm. In some embodiments, an effective average particle size of less than about 100 nm has been achieved. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size less than the effective average, e g , 1000 nm In particularly preferred embodiments essentially all of the particles have a size less than 1000 nm. In some embodiments, essentially all of the particles have a size less than 400 nm

Ratios

The relative amount of therapeutic or diagnostic agent and surface modifier can vary widely and the optimal amount of the surface modifier can depend, for example, upon the particular therapeutic or diagnostic agent and surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, the hydrophilic lipophilic balance (HLB) of the stabilizer, the melting point of the stabilizer, its water solubility, the surface tension of water solutions of the stabilizer, etc The surface modifier preferably is present in an amount of about 0 1–10 mg per square meter surface area of the therapeutic or diagnostic agent. The surface modifier can be present in an amount of 0 1–90%, preferably 20–60% by weight based on the total weight of the dry particle

Diagnosis

A method for diagnostic imaging for use in medical procedures in accordance with this invention comprises intravenously administering to the body of a test subject in need of a diagnostic image an effective contrast producing amount of the diagnostic image contrast composition Thereafter, at least a portion of the body containing the administered contrast agent is exposed to x-rays or a magnetic field to produce an x-ray or magnetic resonance image pattern corresponding to the presence of the contrast agent. The image pattern can then be visualized

Any x-ray visualization technique, preferably, a high contrast technique such as computed tomography, can be applied in a conventional manner. Alternatively, the image pattern can be observed directly on an x-ray sensitive phosphor screen-silver halide photographic film combination or by use of a storage phosphor screen

Visualization with a magnetic resonance imaging system can be accomplished with commercially available magnetic imaging systems such as a General Electric 1 5 T Sigma imaging system [1H resonant frequency 63 9 megahertz (MHz)] Commercially available magnetic resonance imaging systems are typically characterized by the magnetic field strength used, with a field strength of 2 0 Tesla as the current maximum and 0 2 Tesla as the current minimum. For a given field strength, each detected nucleus has a characteristic frequency. For example, at a field strength of 1.0 Tesla, the resonance frequency for hydrogen is 42.57 10 MHz; for phosphorus-31 it is 17 24 MHz; and for sodium23 it is 11 26 Mhz

A contrast effective amount of the diagnostic agent containing composition is that amount necessary to provide tissue visualization with, for example, magnetic resonance imaging or x-ray imaging Means for determining a contrast effective amount in a particular subject will depend, as is well known in the art, on the nature of the magnetically reactive material used, the mass of the subject being imaged, the sensitivity of the magnetic resonance or x-ray imaging system and the like

After administration of the compositions, the subject is maintained for a time period sufficient for the administered compositions to be distributed throughout the subject and enter the tissues of the mammal Typically, a sufficient time period is from about 20 minutes to about 90 minutes and, preferably from about 20 minutes to about 60 minutes

The invention has been described with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention

What is claimed is:

1. A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising intravenously administering to said mammal an effective amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min, wherein said drug composition comprises:

(a) particles consisting essentially of from about 0 1 to about 99 9% by weight of a crystalline organic drug substance having a solubility in water of less than 10 mg/ml;

(b) a surface modifier adsorbed on the surface of the drug substance in an amount of from about 0 1 to about

99.9% by weight and sufficient to maintain an effective average particle size of from about 50 nm to about 1000 nm; and

(c) a pharmaceutically acceptable carrier therefor.

2. The method of claim 1 wherein the effective average particle size of said drug is from about 50 to about 400 nm.

3. The method of claim 1 wherein said drug is an organic therapeutic substance.

4. The method of claim 1 wherein said drug is a diagnostic agent.

5. A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising:

(a) intravenously administering to said mammal an antihistamine in the amount of from about 5 to about 10 mg/kg of body weight; and

(b) subsequently intravenously administering to said mammal an effective amount of a nanoparticulate drug composition comprising: (1) particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml; and (2) a surface modifier adsorbed on the surface of the drug substance in an amount of from about 99.9 to about 0.1% by weight and sufficient to maintain an effective average particle size of less than about 1000 nm.

6. The method of claim 5 wherein the effective average particle size of said drug is of from about 50 to about 400 nm.

7. The method of claim 5 wherein said drug is an organic therapeutic substance.

8. The method of claim 5 wherein said drug is a diagnostic agent.

9. A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising:

(a) intravenously administering to said mammal a desensitizing amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min; and

(b) intravenously administering an effective amount of said nanoparticulate composition comprising: (1) particles consisting essentially of from about 0.1 to about 99.9% by weight of a crystalline organic drug substance having a solubility in water of less than 10 mg/ml; (2) a surface modifier adsorbed on the surface of the drug substance in an amount sufficient to maintain an effective average particle size of from about 100 to about 1000 nm; and (3) a pharmaceutically acceptable carrier therefor.

10. The method of claim 9 wherein said drug is an organic therapeutic substance.

11. The method of claim 9 wherein said drug is a diagnostic agent.

12. The method of claim 9 wherein the effective average particle size of said drug is of from about 100 to about 400 nm.

13. A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising intravenously administering to said mammal an effective amount of a nanoparticulate drug composition at an infusion rate not exceeding 10 mg/min, wherein said drug composition comprises:

(a) particles having an effective average particle size of from about 50 to about 1000 nm and consisting essentially of from about 0.1 to about 99.9% by weight of an organic drug substance entrapped in from about 99.9 to about 0.1% by weight of liposome or a colloidal polymeric material; and

(b) a pharmaceutically acceptable carrier therefor.

14. The method of claim 13 wherein the effective average particle size of said drug is of from about 50 to about 400 nm.

15. The method of claim 13 wherein said drug is an organic therapeutic substance.

16. The method of claim 13 wherein said drug is a diagnostic agent.

17. A method of administering a nanoparticulate composition to a mammal without eliciting adverse hemodynamic effects comprising:

(a) intravenously administering to said mammal an antihistamine in the amount of from about 5 to about 10 mg/kg of body weight; and

(b) subsequently intravenously administering to said mammal an effective amount of a nanoparticulate drug composition comprising: (1) particles having an effective average particle size of from about 50 to bout 1000 nm and consisting essentially of from about 0.1 to about 99.9% by weight of an organic drug substance entrapped in from about 99.9 to about 0.1% by weight of liposome or a colloidal polymeric material; and (2) a pharmaceutically acceptable carrier therefor.

18. The method of claim 17 wherein the effective average particle size of said drug is of from about 50 to about 400 nm.

19. The method of claim 17 wherein said drug is an organic therapeutic substance.

20. The method of claim 17 wherein said drug is a diagnostic agent.

* * * * *

JS 44 (Rev. 3/99)

CIVIL COVER SHEET

The JS-44 civil cover sheet and the information contained herein neither replace nor supplement the filing and service of pleadings or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet. (SEE INSTRUCTIONS ON THE REVERSE OF THE FORM.)

I. (a) PLAINTIFFS

ÉLAN PHARMACEUTICAL INTERNATIONAL LIMITED

(b) County of Residence of First Listed Plaintiff _____
(EXCEPT IN U.S. PLAINTIFF CASES)

DEFENDANTS

ABRAXIS BIOSCIENCE, INC.

County of Residence of First Listed Defendant _____
(IN U.S. PLAINTIFF CASES ONLY)
NOTE: IN LAND CONDEMNATION CASES, USE THE LOCATION OF THE LAND INVOLVED.

(c) Attorney's (Firm Name, Address, and Telephone Number)

Potter Anderson & Corroon LLP
Hercules Plaza, 6th Floor
1313 N. Market Street, P.O. Box 951
Wilmington, DE 19899-0951

Attorneys (If Known)

II. BASIS OF JURISDICTION (Place an "X" in One Box Only)

- [ ] 1 U.S. Government Plaintiff
- [x] 3 Federal Question (U.S. Government Not a Party)
- [ ] 2 U.S. Government Defendant
- [ ] 4 Diversity (Indicate Citizenship of Parties in Item III)

III. CITIZENSHIP OF PRINCIPAL PARTIES (Place an "X" in One Box for Plaintiff and One Box for Defendant)
(For Diversity Cases Only)

	PTF	DEF		PTF	DEF
Citizen of This State	[ ] 1	[ ] 1	Incorporated *or* Principal Place of Business In This State	[ ] 4	[ ] 4
Citizen of Another State	[ ] 2	[ ] 2	Incorporated *and* Principal Place of Business In Another State	[ ] 5	[ ] 5
Citizen or Subject of a Foreign Country	[ ] 3	[ ] 3	Foreign Nation	[ ] 6	[ ] 6

IV. NATURE OF SUIT (Place an "X" in One Box Only)

CONTRACT
- [ ] 110 Insurance
- [ ] 120 Marine
- [ ] 130 Miller Act
- [ ] 140 Negotiable Instrument
- [ ] 150 Recovery of Overpayment & Enforcement of Judgment
- [ ] 151 Medicare Act
- [ ] 152 Recovery of Defaulted Student Loans (Excl. Veterans)
- [ ] 153 Recovery of Overpayment of Veteran's Benefits
- [ ] 160 Stockholders' Suits
- [ ] 190 Other Contract
- [ ] 195 Contract Product Liability

REAL PROPERTY
- [ ] 210 Land Condemnation
- [ ] 220 Foreclosure
- [ ] 230 Rent Lease & Ejectment
- [ ] 240 Torts to Land
- [ ] 245 Tort Product Liability
- [ ] 290 All Other Real Property

TORTS

PERSONAL INJURY
- [ ] 310 Airplane
- [ ] 315 Airplane Product Liability
- [ ] 320 Assault, Libel & Slander
- [ ] 330 Federal Employers' Liability
- [ ] 340 Marine
- [ ] 345 Marine Product Liability
- [ ] 350 Motor Vehicle
- [ ] 355 Motor Vehicle Product Liability
- [ ] 360 Other Personal Injury

PERSONAL INJURY
- [ ] 362 Personal Injury—Med. Malpractice
- [ ] 365 Personal Injury — Product Liability
- [ ] 368 Asbestos Personal Injury Product Liability

PERSONAL PROPERTY
- [ ] 370 Other Fraud
- [ ] 371 Truth in Lending
- [ ] 380 Other Personal Property Damage
- [ ] 385 Property Damage Product Liability

CIVIL RIGHTS
- [ ] 441 Voting
- [ ] 442 Employment
- [ ] 443 Housing/ Accommodations
- [ ] 444 Welfare
- [ ] 440 Other Civil Rights

PRISONER PETITIONS
- [ ] 510 Motions to Vacate Sentence
- Habeas Corpus:
- [ ] 530 General
- [ ] 535 Death Penalty
- [ ] 540 Mandamus & Other
- [ ] 550 Civil Rights
- [ ] 555 Prison Condition

FORFEITURE/PENALTY
- [ ] 610 Agriculture
- [ ] 620 Other Food & Drug
- [ ] 625 Drug Related Seizure of Property 21 USC
- [ ] 630 Liquor Laws
- [ ] 640 R.R. & Truck
- [ ] 650 Airline Regs.
- [ ] 660 Occupational Safety/Health
- [ ] 690 Other

LABOR
- [ ] 710 Fair Labor Standards Act
- [ ] 720 Labor/Mgmt. Relations
- [ ] 730 Labor/Mgmt Reporting & Disclosure Act
- [ ] 740 Railway Labor Act
- [ ] 790 Other Labor Litigation
- [ ] 791 Empl. Ret. Inc. Security Act

BANKRUPTCY
- [ ] 422 Appeal 28 USC 158
- [ ] 423 Withdrawal 28 USC 157

PROPERTY RIGHTS
- [ ] 820 Copyrights
- [x] 830 Patent
- [ ] 840 Trademark

SOCIAL SECURITY
- [ ] 861 HIA (1395ff)
- [ ] 862 Black Lung (923)
- [ ] 863 DIWC/DIWW (405(g))
- [ ] 864 SSID Title XVI
- [ ] 865 RSI (405(g))

FEDERAL TAX SUITS
- [ ] 870 Taxes (U.S. Plaintiff or Defendant)
- [ ] 871 IRS—Third Party 26 USC 7609

OTHER STATUTES
- [ ] 400 State Reapportionment
- [ ] 410 Antitrust
- [ ] 430 Banks and Banking
- [ ] 450 Commerce/ICC Rates/etc.
- [ ] 460 Deportation
- [ ] 470 Racketeer Influenced and Corrupt Organizations
- [ ] 810 Selective Service
- [ ] 850 Securities/Commodities/ Exchange
- [ ] 875 Customer Challenge 12 USC 3410
- [ ] 891 Agricultural Acts
- [ ] 892 Economic Stabilization Act
- [ ] 893 Environmental Matters
- [ ] 894 Energy Allocation Act
- [ ] 895 Freedom of Information Act
- [ ] 900 Appeal of Fee Determination Under Equal Access to Justice
- [ ] 950 Constitutionality of State Statutes
- [ ] 890 Other Statutory Actions

V. ORIGIN (PLACE AN "X" IN ONE BOX ONLY)

- [x] 1 Original Proceeding
- [ ] 2 Removed from State Court
- [ ] 3 Remanded from Appellate Court
- [ ] 4 Reinstated or Reopened
- [ ] 5 Transferred from another district (specify)
- [ ] 6 Multidistrict Litigation
- [ ] 7 Appeal to District Judge from Magistrate Judgment

VI. CAUSE OF ACTION (Cite the U.S. Civil Statute under which you are filing and write brief statement of cause. Do not cite jurisdictional statutes unless diversity.)

35 U.S.C. § 1, et seq. for patent infringement

VII. REQUESTED IN COMPLAINT: [ ] CHECK IF THIS IS A CLASS ACTION UNDER F.R.C.P. 23 DEMAND $ _____ CHECK YES only if demanded in complaint: JURY DEMAND: [x] Yes [ ] No

VIII. RELATED CASE(S) IF ANY (See instructions): JUDGE _____ DOCKET NUMBER _____

DATE 07/19/2006 SIGNATURE OF ATTORNEY OF RECORD Peter L. Hamm

FOR OFFICE USE ONLY

RECEIPT # _____ AMOUNT _____ APPLYING IFP _____ JUDGE _____ MAG. JUDGE _____

JS 44 Reverse (Rev 12/96)

INSTRUCTIONS FOR ATTORNEYS COMPLETING CIVIL COVER SHEET FORM JS-44

Authority For Civil Cover Sheet

The JS-44 civil cover sheet and the information contained herein neither replaces nor supplements the filings and service of pleading or other papers as required by law, except as provided by local rules of court. This form, approved by the Judicial Conference of the United States in September 1974, is required for the use of the Clerk of Court for the purpose of initiating the civil docket sheet. Consequently, a civil cover sheet is submitted to the Clerk of Court for each civil complaint filed. The attorney filing a case should complete the form as follows:

I. **(a) Plaintiffs-Defendants.** Enter names (last, first, middle initial) of plaintiff and defendant. If the plaintiff or defendant is a government agency, use only the full name or standard abbreviations. If the plaintiff or defendant is an official within a government agency, identify first the agency and then the official, giving both name and title.

(b) County of Residence. For each civil case filed, except U.S. plaintiff cases, enter the name of the county where the first listed plaintiff resides at the time of filing. In U.S. plaintiff cases, enter the name of the county in which the first listed defendant resides at the time of filing. (NOTE: In land condemnation cases, the county of residence of the "defendant" is the location of the tract of land involved.)

(c) Attorneys. Enter the firm name, address, telephone number, and attorney of record. If there are several attorneys, list them on an attachment, noting in this section "(see attachment)"

II. **Jurisdiction.** The basis of jurisdiction is set forth under Rule 8(a), F.R.C.P., which requires that jurisdictions be shown in pleadings. Place an "X" in one of the boxes. If there is more than one basis of jurisdiction, precedence is given in the order shown below.

United States plaintiff. (1) Jurisdiction based on 28 U.S.C. 1345 and 1348. Suits by agencies and officers of the United States, are included here.

United States defendant. (2) When the plaintiff is suing the United States, its officers or agencies, place an "X" in this box.

Federal question. (3) This refers to suits under 28 U.S.C. 1331, where jurisdiction arises under the Constitution of the United States, an amendment to the Constitution, an act of Congress or a treaty of the United States. In cases where the U.S. is a party, the U.S. plaintiff or defendant code takes precedence, and box 1 or 2 should be marked.

Diversity of citizenship. (4) This refers to suits under 28 U.S.C. 1332, where parties are citizens of different states. When Box 4 is checked, the citizenship of the different parties must be checked. (See Section III below; federal question actions take precedence over diversity cases.)

III. **Residence (citizenship) of Principal Parties.** This section of the JS-44 is to be completed if diversity of citizenship was indicated above. Mark this section for each principal party.

IV. **Nature of Suit.** Place an "X" in the appropriate box. If the nature of suit cannot be determined, be sure the cause of action, in Section IV below, is sufficient to enable the deputy clerk or the statistical clerks in the Administrative Office to determine the nature of suit. If the cause fits more than one nature of suit, select the most definitive.

V. **Origin.** Place an "X" in one of the seven boxes.

Original Proceedings. (1) Cases which originate in the United States district courts.

Removed from State Court. (2) Proceedings initiated in state courts may be removed to the district courts under Title 28 U.S.C., Section 1441. When the petition for removal is granted, check this box.

Remanded from Appellate Court. (3) Check this box for cases remanded to the district court for further action. Use the date of remand as the filing date.

Reinstated or Reopened. (4) Check this box for cases reinstated or reopened in the district court. Use the reopening date as the filing date.

Transferred from Another District. (5) For cases transferred under Title 28 U.S.C. Section 1404(a). Do not use this for within district transfers or multidistrict litigation transfers.

Multidistrict Litigation. (6) Check this box when a multidistrict case is transferred into the district under authority of Title 28 U.S.C. Section 1407. When this box is checked, do not check (5) above.

Appeal to District Judge from Magistrate Judgment. (7) Check this box for an appeal from a magistrate judge's decision.

VI. **Cause of Action.** Report the civil statute directly related to the cause of action and give a brief description of the cause.

VII. **Requested in Complaint.** Class Action. Place an "X" in this box if you are filing a class action under Rule 23, F.R.Cv.P.

Demand. In this space enter the dollar amount (in thousands of dollars) being demanded or indicate other demand such as a preliminary injunction.

Jury Demand. Check the appropriate box to indicate whether or not a jury is being demanded.

VIII. **Related Cases.** This section of the JS-44 is used to reference related pending cases if any. If there are related pending cases, insert the docket numbers and the corresponding judge names for such cases.

Date and Attorney Signature. Date and sign the civil cover sheet.

AO FORM 85 RECEIPT (REV. 9/04)

United States District Court for the District of Delaware

Civil Action No. 06 - 438

ACKNOWLEDGMENT OF RECEIPT FOR AO FORM 85

NOTICE OF AVAILABILITY OF A UNITED STATES MAGISTRATE JUDGE TO EXERCISE JURISDICTION

I HEREBY ACKNOWLEDGE RECEIPT OF 2 COPIES OF AO FORM 85.

7/19/06
(Date forms issued)

Robert Testa
(Signature of Party or their Representative)

Robert Testa
(Printed name of Party or their Representative)

Note: Completed receipt will be filed in the Civil Action