IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

ELAN PHARMA INTERNATIONAL LIMITED, Plaintiff, v. ABRAXIS BIOSCIENCE, INC., Defendant.	)	C.A. No. 06-438-GMS **REDACTED – PUBLIC VERSION**

APPENDIX OF EXHIBITS TO DEFENDANT ABRAXIS BIOSCIENCE, INC.'S ANSWERING BRIEF IN OPPOSITION TO ELAN'S CONDITIONAL MOTION FOR NEW TRIAL

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JX006	NDA 21660 - II. Drug Product--Components and Composition	04/25/2003	C 1-136
JX011	Email - Neil Desai to Zachary Yim re nanoparticles comparison with competitors	05/09/2005	C 137-138
JX020	Letter – G. Liversidge to Neil Desai re enclosing Nanosystems presentation and patents	08/28/1996	C 139-230
JX021	Excerpts from Laboratory Notebook 24 (Neil Desai)	09/04/1996	C 231-235
DX041	Memorandum from E. Gresham from R. Selvaraj re XRPD of ABI-007 Samples Micron Inc. Analytical Services Report #27872 Micron Inc. Analytical Services Report #28217 Micron Inc. Analytical Services Report #28757 Micron Inc. Analytical Services Report #29007 Micron Inc. Analytical Services Report #29101 Micron Inc. Analytical Services Report #33063 Micron Inc. Analytical Services Report #33370 Abraxis Research Report PD06-NP/N-004 titled, "Comparative Study on Characterization Results of Abraxane Commercial and Stability Lots" Technical Report # PD03-NP/P-020 titled, "Paclitaxel and Human Albumin Assays in Supernatant Liquid and Pellet of ABI-007 Reconstituted Suspension" Laboratory Notebook 23938 (Maureen Connell)	03/21/2002 03/28/2002 06/21/2002 10/21/2002 12/13/2002 01/14/2003 02/27/2006 04/21/2006 00/00/2002	C 236-481
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CERTIFICATE OF SERVICE

I, Karen E. Keller, Esquire, hereby certify that on July 25, 2008, I caused to be electronically filed a true and correct copy of the foregoing document with the Clerk of the Court using CM/ECF, which will send notification that such filing is available for viewing and downloading to the following counsel of record:

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United States Patent [19]

Desai et al.

[11] Patent Number: 5,916,596

[45] Date of Patent: Jun. 29, 1999

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

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

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

[21] Appl. No.: **08/720,756**

[22] Filed: **Oct. 1, 1996**

Related U.S. Application Data

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

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

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

[58] **Field of Search** 424/489, 422, 424/423, 475, 9.1, 9.3, 9.32, 450, 400

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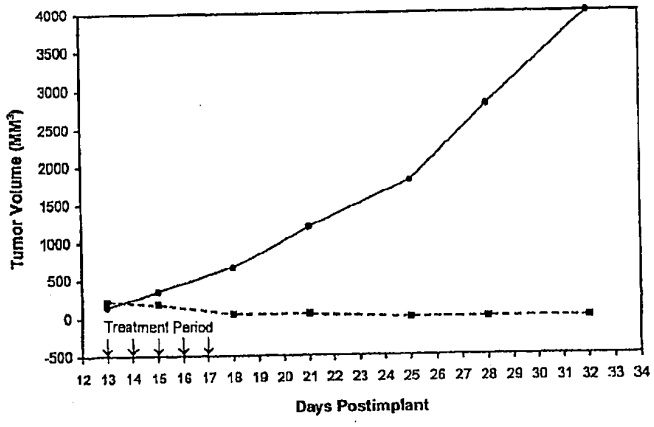
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[57] **ABSTRACT**

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

31 Claims, 2 Drawing Sheets



Case No. 06-438-GMS
DX-605

ABRX 0221168

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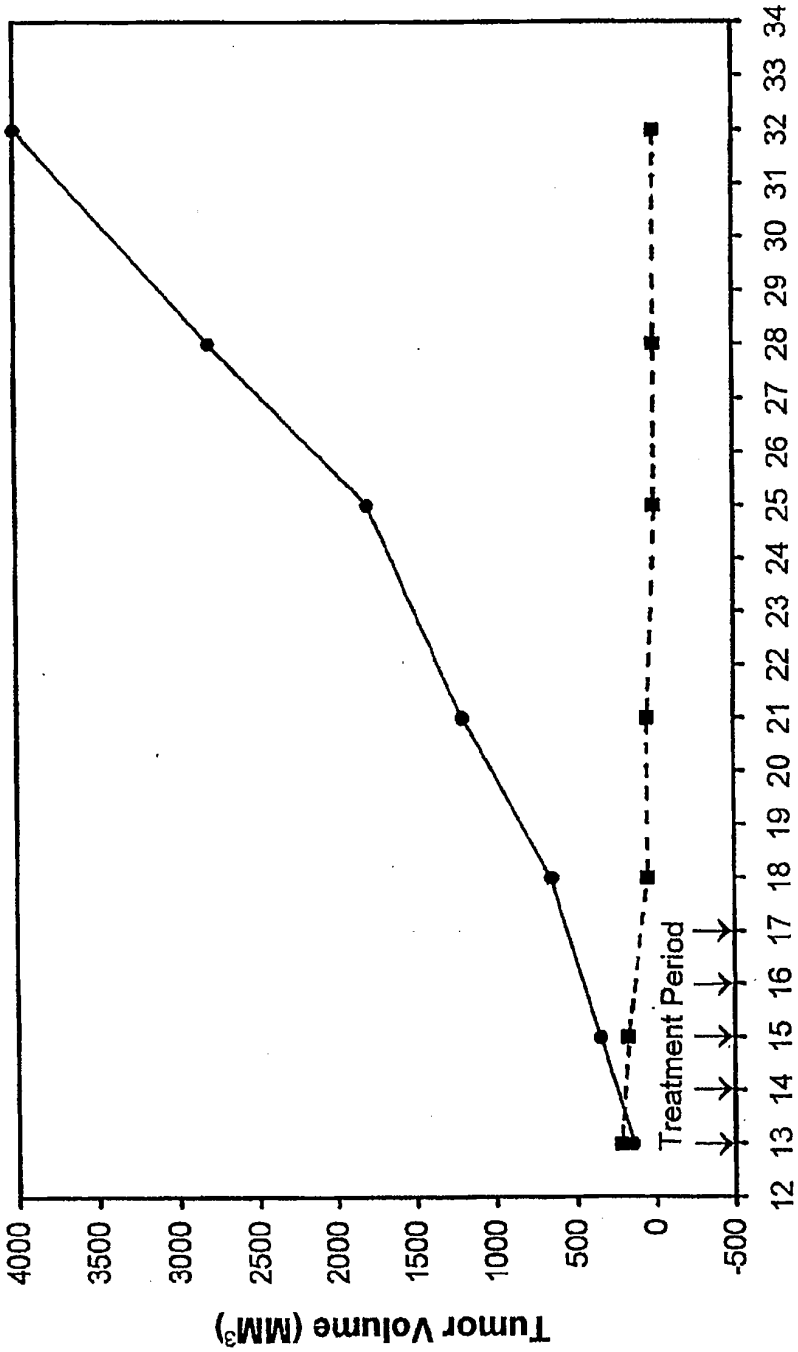


Figure 1

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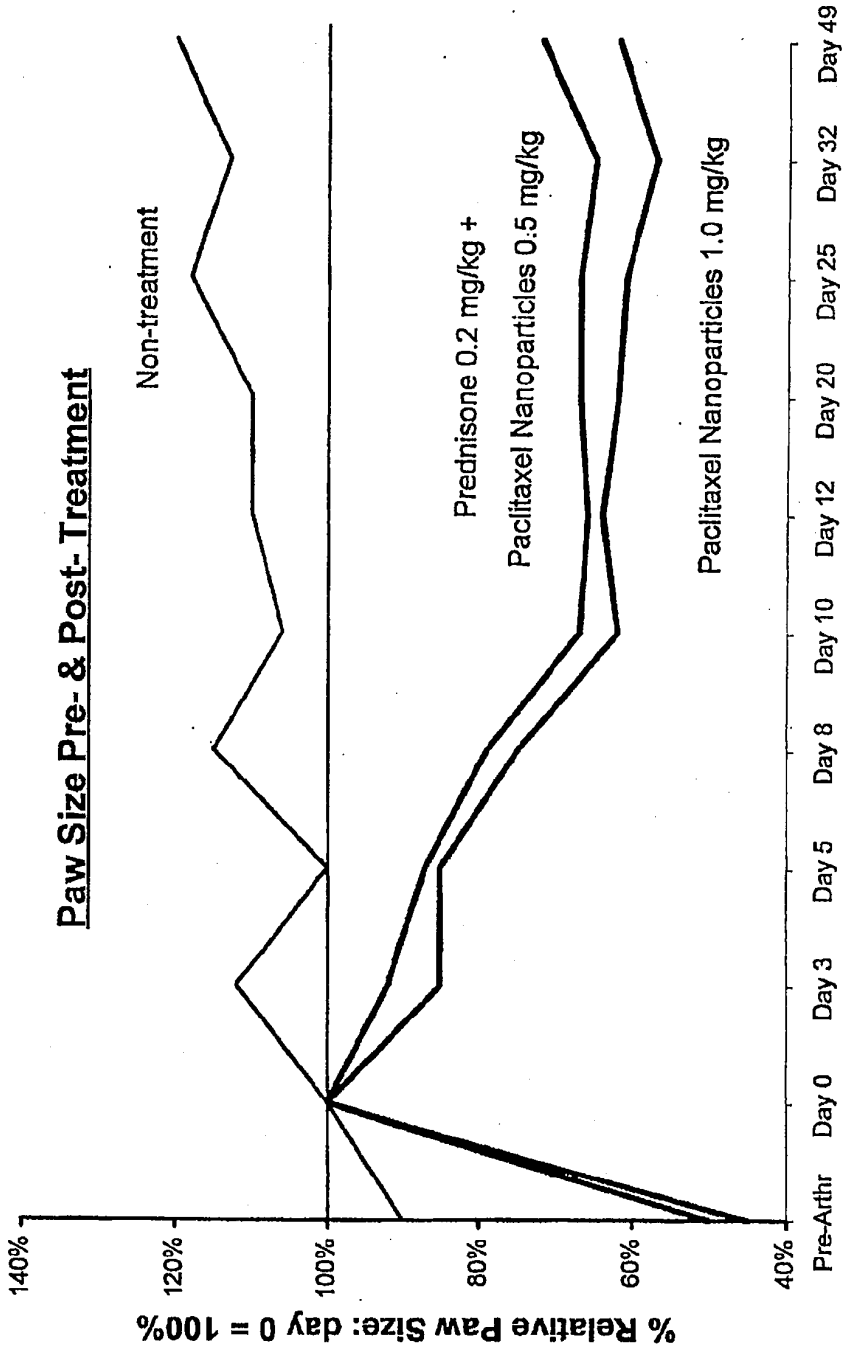


Figure 2

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

RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Ser. No. 08/412,726, filed Mar. 29, 1995, now issued as U.S. Pat. No. 5,560,933, which is, in turn, a divisional of U.S. Ser. No. 08/023,698, filed Feb. 22, 1993, now issued as U.S. Pat. No. 5,439,686, the entire contents of both of which are hereby incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

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

BACKGROUND OF THE INVENTION

Intravenous drug delivery permits rapid and direct equilibration with the blood stream which carries the medication to the rest of the body. To avoid the peak serum levels which are achieved within a short time after intravascular injection, administration of drugs carried within stable carriers would allow gradual release of the drugs inside the intravascular compartment following a bolus intravenous injection of the therapeutic nanoparticles.

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

Microparticles and foreign bodies present in the blood are generally cleared from the circulation by the "blood filtering organs", namely the spleen, lungs and liver. The particulate matter contained in normal whole blood comprises red blood cells (typically 8 microns in diameter), white blood cells (typically 6–8 microns in diameter), and platelets (typically 1–3 microns in diameter). The microcirculation in most

organs and tissues allows the free passage of these blood cells. When microthrombii (blood clots) of size greater than 10–15 microns are present in circulation, a risk of infarction or blockage of the capillaries results, leading to ischemia or oxygen deprivation and possible tissue death. Injection into the circulation of particles greater than 10–15 microns in diameter, therefore, must be avoided. A suspension of particles less than 7–8 microns, is however, relatively safe and has been used for the delivery of pharmacologically active agents in the form of liposomes and emulsions, nutritional agents, and contrast media for imaging applications.

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

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

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

Taxol, a naturally occurring diterpenoid, has been shown to have significant antineoplastic and anticancer effects in drug-refractory ovarian cancer. Taxol has shown excellent antitumor activity in a wide variety of tumor models such as the B16 melanoma, L1210 leukemias, MX-1 mammary tumors, and CS-1 colon tumor xenografts. Several recent press releases have termed taxol as the new anticancer wonder-drug. Indeed, taxol has recently been approved by the Federal Drug Administration for treatment of ovarian

cancer. The poor aqueous solubility of taxol, however, presents a problem for human administration. Indeed, the delivery of drugs that are inherently insoluble or poorly soluble in an aqueous medium can be seriously impaired if oral delivery is not effective. Accordingly, currently used taxol formulations require a cremaphor to solubilize the drug. The human clinical dose range is 200–500 mg. This dose is dissolved in a 1:1 solution of ethanol:cremaphor and diluted to one liter of fluid given intravenously. The cremaphor currently used is polyethoxylated castor oil.

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

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

Protein microspheres have been reported in the literature as carriers of pharmacological or diagnostic agents. Microspheres of albumin have been prepared by either heat denaturation or chemical crosslinking. Heat denatured microspheres are produced from an emulsified mixture (e.g., albumin, the agent to be incorporated, and a suitable oil) at temperatures between 100° C. and 150° C. The microspheres are then washed with a suitable solvent and stored. Leucuta et al. (*International Journal of Pharmaceutics* 41:213–217 (1988)) describe the method of preparation of heat denatured microspheres.

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

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

One conventional method for manufacturing drug-containing nanoparticles comprises dissolving polylactic acid (or other biocompatible, water insoluble polymers) in a water-immiscible solvent (such as methylene chloride or other chlorinated, aliphatic, or aromatic solvent), dissolving the pharmaceutically active agent in the polymer solution, adding a surfactant to the oil phase or the aqueous phase, forming an oil-in-water emulsion by suitable means, and evaporating the emulsion slowly under vacuum. If the oil droplets are sufficiently small and stable during evaporation, a suspension of the polymer in water is obtained. Since the drug is initially present in the polymer solution, it is possible to obtain by this method, a composition in which the drug molecules are entrapped within particles composed of a polymeric matrix. The formation of microspheres and nanoparticles by using the solvent evaporation method has been reported by several researchers (see, for example, Tice and Gilley, in *Journal of Controlled Release* 2:343–352 (1985); Bodmeier and McGinity, in *Int. J. Pharmaceutics* 43:179 (1988); Cavalier et al., in *J. Pharm. Pharmacol.* 38:249 (1985); and D'Souza et al., WO 94/10980) while using various drugs.

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

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

Another process which is based on the solvent evaporation method comprises dissolving the drug in a hydrophobic solvent (e.g., toluene or cyclohexane), without any polymer dissolved in the organic solvent, adding a conventional surfactant to the mixture as an emulsifier, forming an oil-in-water emulsion, and then evaporating the solvent to obtain dry particles of the drug (see, for example, Sjostrom et al., in *J. Dispersion Science and Technology* 15:89–117 (1994)). Upon removal of the nonpolar solvent, precipitation of the drug inside the solvent droplets occurs, and submicron particles are obtained.

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

Formation of submicron particles has also been achieved by a precipitation process, as described by Calvo et al. in *J.*

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

BRIEF DESCRIPTION OF THE INVENTION

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

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

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

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

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

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

The invention further provides a method for the reproducible formation of unusually small nanoparticles (less than 200 nm diameter), which can be sterile-filtered through a 0.22 micron filter. This is achieved by addition of a water soluble solvent (e.g. ethanol) to the organic phase and by carefully selecting the type of organic phase, the phase fraction and the drug concentration in the organic phase. The ability to form nanoparticles of a size that is filterable by 0.22 micron filters is of great importance and significance, since formulations which contain a significant amount of any protein (e.g., albumin), cannot be sterilized by conventional methods such as autoclaving, due to the heat coagulation of the protein.

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

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

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

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

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

In accordance with the present invention, there are also provided submicron particles in powder form, which can easily be reconstituted in water or saline. The powder is obtained after removal of water by lyophilization. Human serum albumin serves as the structural component of invention nanoparticles, and also as a cryoprotectant and reconstitution aid. The preparation of particles filterable through a 0.22 micron filter according to the invention method as described herein, followed by drying or lyophilization, produces a sterile solid formulation useful for intravenous injection.

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

While it is recognized that particles produced according to the invention can be either crystalline, amorphous, or a mixture thereof, it is generally preferred that the drug be present in the formulation in an amorphous form. This would lead to greater ease of dissolution and absorption, resulting in better bioavailability.

BRIEF DESCRIPTION OF THE FIGURES

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

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

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided methods for the preparation of substantially water insoluble pharmacologically active agents for in vivo delivery, said method comprising:

subjecting a mixture comprising:

an organic phase containing said pharmacologically active agent dispersed therein, and

aqueous medium containing biocompatible polymer,

wherein said mixture contains substantially no surfactants,

in a high pressure homogenizer at a pressure in the range of about 3,000 up to 30,000 psi. Optionally, the organic and/or aqueous phases are thereafter removed from the mixture after having been subjected to high shear conditions.

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

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

wherein said protein coating has free protein associated therewith,

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

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

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

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

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

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

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

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

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

Thus, in accordance with the present invention, a pharmacologically active agent is dissolved in a suitable solvent (e.g., chloroform, methylene chloride, ethyl acetate, ethanol, tetrahydrofuran, dioxane, acetonitrile, acetone, dimethyl sulfoxide, dimethyl formamide, methyl pyrrolidinone, or the like, as well as mixtures of any two or more thereof). Additional solvents contemplated for use in the practice of the present invention include soybean oil, coconut oil, olive oil, safflower oil, cotton seed oil, sesame oil, orange oil, limonene oil, C1–C20 alcohols, C2–C20 esters, C3–C20 ketones, polyethylene glycols, aliphatic hydrocarbons, aromatic hydrocarbons, halogenated hydrocarbons and combinations thereof.

Unlike conventional methods for nanoparticle formation, a polymer (e.g. polylactic acid) is not dissolved in the solvent. The oil phase employed in the preparation of invention compositions contains only the pharmacologically active agent dissolved in solvent.

Next, a protein (e.g., human serum albumin) is added (into the aqueous phase) to act as a stabilizing agent for the formation of stable nanodroplets. Protein is added at a concentration in the range of about 0.05 to 25 % (w/v), more preferably in the range of about 0.5%–5% (w/v). Unlike conventional methods for nanoparticle formation, no surfactant (e.g. sodium lauryl sulfate, lecithin, tween 80, pluronic F-68 and the like) is added to the mixture.

Next, an emulsion is formed by homogenization under high pressure and high shear forces. Such homogenization is conveniently carried out in a high pressure homogenizer, typically operated at pressures in the range of about 3,000 up to 30,000 psi. Preferably, such processes are carried out at pressures in the range of about 6,000 up to 25,000 psi. The resulting emulsion comprises very small nanodroplets of the nonaqueous solvent (containing the dissolved pharmacologically active agent) and very small nanodroplets of the protein stabilizing agent. Acceptable methods of homogenization include processes imparting high shear and cavitation such as high pressure homogenization, high shear mixers, sonication, high shear impellers, and the like.

Finally, the solvent is evaporated under reduced pressure to yield a colloidal system composed of protein coated nanoparticles of pharmacologically active agent and protein. Acceptable methods of evaporation include the use of rotary evaporators, falling film evaporators, spray driers, freeze driers, and the like.

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

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

In order to obtain sterile-filterable particles (i.e., particles <200 nm), the pharmacologically active agent is initially dissolved in a substantially water immiscible organic solvent (e.g., a solvent having less than about 5% solubility in water, such as, for example, chloroform) at high concentration, thereby forming an oil phase containing the pharmacologically active agent. Suitable solvents are set forth above. Unlike conventional methods for nanoparticle formation, a polymer (e.g. polylactic acid) is not dissolved in the solvent. The oil phase employed in the process of the present invention contains only the pharmacologically active agent dissolved in solvent.

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

Next, human serum albumin or any other suitable stabilizing agent as described above is dissolved in aqueous media. This component acts as a stabilizing agent for the formation of stable nanodroplets. Optionally, a sufficient amount of the first organic solvent (e.g. chloroform) is dissolved in the aqueous phase to bring it close to the saturation concentration. A separate, measured amount of the organic phase (which now contains the pharmacologically active agent, the first organic solvent and the second organic solvent) is added to the saturated aqueous phase, so that the phase fraction of the organic phase is between about 0.5%–015% v/v, and more preferably between 1% and 8% v/v.

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

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

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

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

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

- analgesics/antipyretics (e.g., aspirin, acetaminophen, ibuprofen, naproxen sodium, buprenorphine hydrochloride, propoxyphene hydrochloride, propoxyphene napsylate, meperidine hydrochloride, hydromorphone hydrochloride, morphine sulfate, oxycodone hydrochloride, codeine phosphate, dihydrocodeine bitartrate, pentazocine hydrochloride, hydrocodone bitartrate, levorphanol tartrate, diflunisal, trolamine salicylate, nalbuphine hydrochloride, mefenamic acid, butorphanol tartrate, choline salicylate, butalbital, phenyltoloxamine citrate, diphenhydramine citrate, methotrimeprazine, cinnamedrine hydrochloride, meprobamate, and the like);
- anesthetics (e.g., cyclopropane, enflurane, halothane, isoflurane, methoxyflurane, nitrous oxide, propofol, and the like);
- antiasthamatics (e.g., Azelastine, Ketotifen, Traxanox, and the like);
- antibiotics (e.g., neomycin, streptomycin, chloramphenicol, cephalosporin, ampicillin, penicillin, tetracycline, and the like);
- antidepressants (e.g., nefopam, oxypertine, doxepin hydrochloride, amoxapine, trazodone hydrochloride, amitriptyline hydrochloride, maprotiline hydrochloride, phenelzine sulfate, desipramine hydrochloride, nortriptyline hydrochloride, tranylcypromine sulfate, fluoxetine hydrochloride, doxepin hydrochloride, imipramine hydrochloride, imipramine pamoate, nortriptyline, amitriptyline hydrochloride, isocarboxazid, desipramine hydrochloride, trimipramine maleate, protriptyline hydrochloride, and the like);
- antidiabetics (e.g., biguanides, hormones, sulfonylurea derivatives, and the like);
- antifungal agents (e.g., griseofulvin, keloconazole, amphotericin B, Nystatin, candicidin, and the like);
- antihypertensive agents (e.g., propanolol, propafenone, oxyprenolol, Nifedipine, reserpine, trimethaphan camsylate, phenoxybenzamine hydrochloride, pargyline hydrochloride, deserpidine, diazoxide, guanethidine monosulfate, minoxidil, rescinnamine, sodium nitroprusside, rauwolfia serpentina, alseroxylon, phentolamine mesylate, reserpine, and the like);
- anti-inflammatories (e.g., (non-steroidal) indomethacin, naproxen, ibuprofen, ramifenazone, piroxicam, (steroidal) cortisone, dexamethasone, fluazacort, hydrocortisone, prednisolone, prednisone, and the like);
- antineoplastics (e.g., adriamycin, cyclophosphamide, actinomycin, bleomycin, duanorubicin, doxorubicin, epirubicin, mitomycin, methotrexate, fluorouracil, carboplatin, carmustine (BCNU), methyl-CCNU, cisplatin, etoposide, interferons, camptothecin and derivatives thereof, phenesterine, taxol and derivatives thereof, taxotere and derivatives thereof, vinblastine, vincristine, tamoxifen, etoposide, piposulfan, and the like);
- antianxiety agents (e.g., lorazepam, buspirone hydrochloride, prazepam, chlordiazepoxide hydrochloride, oxazepam, clorazepate dipotassium, diazepam, hydroxyzine pamoate, hydroxyzine hydrochloride, alprazolam, droperidol, halazepam, chlormezanone, dantrolene, and the like);
- immunosuppressive agents (e.g., cyclosporine, azathioprine, mizoribine, FK506 (tacrolimus), and the like); antimigraine agents (e.g., ergotamine tartrate, propanolol hydrochloride, isometheptene mucate, dichloralphenazone, and the like);
- sedatives/hypnotics (e.g., barbiturates (e.g., pentobarbital, pentobarbital sodium, secobarbital sodium), benzodiazapines (e.g., flurazepam hydrochloride, triazolam, tomazeparm, midazolam hydrochloride, and the like);
- antianginal agents (e.g., beta-adrenergic blockers, calcium channel blockers (e.g., nifedipine, diltiazem hydrochloride, and the like), nitrates (e.g., nitroglycerin, isosorbide dinitrate, pentaerythritol tetranitrate, erythrityl tetranitrate, and the like));
- antipsychotic agents (e.g., haloperidol, loxapine succinate, loxapine hydrochloride, thioridazine, thioridazine hydrochloride, thiothixene, fluphenazine hydrochloride, fluphenazine decanoate, fluphenazine enanthate, trifluoperazine hydrochloride, chlorpromazine hydrochloride, perphenazine, lithium citrate, prochlorperazine, and the like);
- antimanic agents (e.g., lithium carbonate);
- antiarrhythmics (e.g., bretylium tosylate, esmolol hydrochloride, verapamil hydrochloride, amiodarone, encainide hydrochloride, digoxin, digitoxin, mexiletine hydrochloride, disopyramide phosphate, procainamide hydrochloride, quinidine sulfate, quinidine gluconate, quinidine polygalacturonate, flecainide acetate, tocainide hydrochloride, lidocaine hydrochloride, and the like);
- antiarthritic agents (e.g., phenylbutazone, sulindac, penicillamine, salsalate, piroxicam, azathioprine, indomethacin, meclofenamate sodium, gold sodium thiomalate, ketoprofen, auranofin, aurothioglucose, tolmetin sodium, and the like);
- antigout agents (e.g., colchicine, allopurinol, and the like);
- anticoagulants (e.g., heparin, heparin sodium, warfarin sodium, and the like);
- thrombolytic agents (e.g., urokinase, streptokinase, altoplase, and the like);
- antifibrinolytic agents (e.g., aminocaproic acid);
- hemorheologic agents (e.g., pentoxifylline);
- antiplatelet agents (e.g., aspirin, empirin, ascriptin, and the like);
- anticonvulsants (e.g., valproic acid, divalproate sodium, phenytoin, phenytoin sodium, clonazepam, primidone,

phenobarbitol, phenobarbitol sodium, carbamazepine, amobarbital sodium, methsuximide, metharbital, mephobarbital, mephenytoin, phensuximide, paramethadione, ethotoin, phenacemide, secobarbitol sodium, clorazepate dipotassium, trimethadione, and the like);

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

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

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

antibacterial agents (e.g., amikacin sulfate, aztreonam, chloramphenicol, chloramphenicol palmitate, chloramphenicol sodium succinate, ciprofloxacin hydrochloride, clindamycin hydrochloride, clindamycin palmitate, clindamycin phosphate, metronidazole, metronidazole hydrochloride, gentamicin sulfate, lincomycin hydrochloride, tobramycin sulfate, vancomycin hydrochloride, polymyxin B sulfate, colistimethate sodium, colistin sulfate, and the like);

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

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

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

bronchodialators (e.g., sympathomimetics (e.g., epinephrine hydrochloride, metaproterenol sulfate, terbutaline sulfate, isoetharine, isoetharine mesylate, isoetharine hydrochloride, albuterol sulfate, albuterol, bitolterol, mesylate isoproterenol hydrochloride, terbutaline sulfate, epinephrine bitartrate, metaproterenol sulfate, epinephrine, epinephrine bitartrate), anticholinergic agents (e.g., ipratropium bromide), xanthines (e.g., aminophylline, dyphylline, metaproterenol sulfate, aminophylline), mast cell stabilizers (e.g., cromolyn sodium), inhalant corticosteroids (e.g., flurisolidebeclomethasone dipropionate, beclomethasone dipropionate monohydrate), salbutamol, beclomethasone dipropionate (BDP), ipratropium bromide, budesonide, ketotifen, salmeterol, xinafoate, terbutaline sulfate, triamcinolone, theophylline, nedocromil sodium, metaproterenol sulfate, albuterol, flunisolide, and the like);

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

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

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

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

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

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

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

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

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

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

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

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

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

Proteins contemplated for use as stabilizing agents in accordance with the present invention include albumins (which contain 35 cysteine residues), immunoglobulins, caseins, insulins (which contain 6 cysteines), hemoglobins (which contain 6 cysteine residues per $\alpha_2\beta_2$ unit), lysozymes (which contain 8 cysteine residues), immunoglobulins, α-2-macroglobulin, fibronectins, vitronectins, fibrinogens, lipases, and the like. Proteins, peptides, enzymes, antibodies and combinations thereof, are general classes of stabilizers contemplated for use in the present invention.

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

Similarly, synthetic polypeptides containing cysteine residues are also good candidates for formation of a shell about the substantially water insoluble pharmacologically active agents. In addition, polyvinyl alcohol, polyhydroxyethyl methacrylate, polyacrylic acid, polyethyloxazoline, polyacrylamide, polyvinyl pyrrolidinone, and the like, are good candidates for chemical modification (for example, by the introduction of sulfhydryl and/or disulfide linkages) and shell formation (by causing the crosslinking thereof). Thus, for example, contemplated for use in the practice of the present invention are such materials as synthetic polyamino acids containing cysteine residues and/or disulfide groups; polyvinyl alcohol modified to contain free sulfhydryl groups and/or disulfide groups; polyhydroxyethyl methacrylate modified to contain free sulfhydryl groups and/or disulfide groups; polyacrylic acid modified to contain free sulfhydryl groups and/or disulfide groups; polyethyloxazoline modified to contain free sulfhydryl groups and/or disulfide groups; polyacrylamide modified to contain free sulfhydryl groups and/or disulfide groups; polyvinyl pyrrolidinone modified to contain free sulfhydryl groups and/or disulfide groups; polyalkylene glycols modified to contain free sulfhydryl groups and/or disulfide groups; polylactides, polyglycolides, polycaprolactones, or copolymers thereof, modified to contain free sulfhydryl groups and/or disulfide groups; as well as mixtures of any two or more thereof.

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

Especially preferred combinations of organic media contemplated for use in the practice of the present invention typically have a boiling point of no greater than about 200° C., and include volatile liquids such as dichloromethane, chloroform, ethyl acetate, benzene, and the like (i.e., solvents that have a high degree of solubility for the pharmacologically active agent, and are soluble in the other organic medium employed), along with a higher molecular weight (less volatile) organic medium. When added to the other organic medium, these volatile additives help to drive the solubility of the pharmacologically active agent into the organic medium. This is desirable since this step is usually time consuming. Following dissolution, the volatile component may be removed by evaporation (optionally under vacuum).

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

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

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

EXAMPLE 1

Preparation of Nanoparticles by High Pressure Homogenization

30 mg paclitaxel is dissolved in 3.0 ml methylene chloride. The solution was added to 27.0 ml of human serum albumin solution (1% w/v). The mixture was homogenized for 5 minutes at low RPM (Vitris homogenizer, model:

Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification was performed at 9000–18,000 psi while recycling the emulsion for at least 5 cycles. The resulting system was transferred into a Rotary evaporator, and methylene chloride was rapidly removed at 40° C., at reduced pressure (30 mm Hg), for 20–30 minutes. The resulting dispersion was translucent, and the typical diameter of the resulting paclitaxel particles was 160–220 (Z-average, Malvern Zetasizer).

The dispersion was further lyophilized for 48 hrs. without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

EXAMPLE 2

Preparation of Nanoparticles by Sonication

The purpose of this example is to demonstrate the formation of nanoparticles of Paclitaxel by using cavitation and high shear forces during a sonication process. Thus, 20 mg paclitaxel is dissolved in 1.0 ml methylene chloride. The solution is added to 4.0 ml of human serum abumin solution (5% w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I.Q.) in order to form a crude emulsion, and then transferred into a 40 kHz sonicator cell. The sonicator is performed at 60–90% power at 0 degree for 1 min (550 Sonic Dismembrator). The mixture is transferred into a Rotary evaporator, and methylene chloride is rapidly removed at 40° C., at reduced pressure (30 mm Hg), for 20–30 minutes. The typical diameter of the resulting paclitaxel particles was 350–420 nm (Z-average, Malvern Zetasizer).

The dispersion was further lyophilized for 48 hrs. without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

EXAMPLE 3

Use of Conventional Surfactants and Proteins Results in formation of Large crystals

The following example demonstrates the effect of adding surfactants which are used in the conventional solvent evaporation method. A series of experiments was conducted employing a similar procedure to that described in Example 1, but a surfactant such as Tween 80 (1% to 10%) is added to the organic solvent. It was found that after removal of the methylene chloride, a large number of paclitaxel crystals is obtained having an average size of 1–2 micron, as viewed by light microscopy and under polarized light. The crystals grow within a few hours to form very large needle-like crystals, with a size in the range of about 5–15 micron. A similar phenomenon is observed with other commonly used surfactants, such as Pluronic F-68, Pluronic F 127, Cremophor EL and Brij 58.

From these results it can be concluded that the conventional solvent evaporation method utilizing conventional surfactants in combination with a protein such as albumin is not suitable for the formation of submicron drug particles (e.g. Paclitaxel) without a polymeric core, while using a polar solvent (e.g., methylene chloride).

EXAMPLE 4

Use of Conventional Surfactants Alone Results in formation of Large crystals

This example demonstrates that it is not possible to form nanoparticles while using conventional surfactants, without a polymeric core material, with pharmacologically active agents which are soluble in polar, water immiscible solvents (e.g. chloroform).

30 mg Taxol is dissolved in 0.55 ml chloroform and 0.05 ml ethanol. The solution is added to 29.4 ml of Tween 80 solution (1% w/v), which is presaturated with 1% chloroform. The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000–18,000 psi while recycling the emulsion for at least 6 cycles. The resulting system was transferred into a Rotary evaporator, and the chloroform was rapidly removed at 40° C., at reduced pressure (30 mm Hg), for 15–30 minutes. The resulting dispersion was opaque, and contained large needle-like crystals of the drug. The initial size of the crystals (observed also by polarized light), was 0.7–5 micron. Storage of the dispersion for several hours at room temperature led to further increase in crystal size, and ultimately to precipitation.

EXAMPLE 5

Preparation of Less than 200 nm Sterile-Filterable Nanoparticles

This example describes the process by which sterile-filterable drug particles can be obtained. Thus, 30 mg Taxol is dissolved in 0.55 ml chloroform and 0.05 ml ethanol. The solution is added to 29.4 ml of human serum abumin solution (1% w/v), which is presaturated with 1% chloroform. The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000–18,000 psi while recycling the emulsion for at least 6 cycles. The resulting system is transferred into a Rotary evaporator, and the chloroform is rapidly removed at 40° C., at reduced pressure (30 mm Hg), for 15–30 minutes. The resulting dispersion is translucent, and the typical diameter of the resulting Taxol particles is 140–160 nm (Z-average, Malvern Zeta Sizer). The dispersion is filtered through a 0.22 micron filter (Millipore), without any significant change in turbidity, or particle size. HPLC analysis of the Taxol content revealed that more than 97% of the Taxol was recovered after filtration, thus providing a sterile Taxol dispersion.

The sterile dispersion was further lyophilized for 48 hrs. without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

EXAMPLE 6

Preparation of Less than 200 nm Sterile-Filterable Nanoparticles

This example describes the process by which sterile-filterable drug particles can be obtained. Thus, 225 mg Taxol is dissolved in 2.7 ml chloroform and 0.3 ml ethanol. The solution is added to 97 ml of human serum abumin solution (3% w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000–18,000 psi while recycling the emulsion for at least 6 cycles. The resulting system is transferred into a

Rotary evaporator, and the chloroform is rapidly removed at 400° C., at reduced pressure (30 mm Hg), for 15–30 minutes. The resulting dispersion is translucent, and the typical diameter of the resulting Taxol particles is 140–160 nm (Z-average, Malvern Zeta Sizer). The dispersion is filtered through a 0.22 micron filter (Sartorius, sartobran 300), without any significant change in turbidity, or particle size. HPLC analysis of the Taxol content typically revealed that 70–100% of the Taxol could be recovered after filtration, depending on the conditions employed. Thus, a sterile Taxol dispersion was obtained.

The sterile dispersion was aseptically filled into sterile glass vials and lyophilized without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

EXAMPLE 7

Effect of Phase Fraction of Organic Solvent on Particle size

The following example demonstrates the importance of having an unusually low phase fraction of the organic solvent in the system.

Thus, a series of experiments was conducted following a similar procedure to that described for Example 5, except the phase fraction of the organic solvent was altered, and the ethanol content maintained at 10% v/v in the organic phase. It was found that increasing the phase fraction led to a significant increase in particle size: at 4% v/v phase fraction (above the saturation concentration, or 5% v/v total chloroform concentration) the resulting particles have a diameter of 250 nm; at 3% v/v phase fraction, the particles have a 200 nm diameter, and at 2% v/v phase fraction, the particles have a 150 nm diameter.

Clearly, only the particles prepared at very low phase fraction could be sterile-filtered.

EXAMPLE 8

Effect of Drug Concentration on Particle Size

The role of drug concentration in the organic phase is demonstrated in the following example. Two experiments were performed in which the Taxol concentration in the organic phase was 50 mg/ml or 75 mg/ml, while all other parameters were the same as described in Example 3. It was found that the low drug concentration yielded particles having a diameter of about 150 nm, while those prepared at the higher drug loading were smaller, i.e., 130–138 nm. When a similar experiment was performed, but with an ethanol concentration in the organic phase of about 50%, a similar trend was observed, i.e., particles were 210 nm and 156 nm in diameter, for 25 mg/ml and 50 mg/ml drug concentration, respectively.

These findings directly contradict those reported by Sjostrom et al., supra, for the formation of nanoparticles in presence of surfactants.

EXAMPLE 9

Nanoparticle formation of a model Drug

30 mg Isoreserpine (a model drug) is dissolved in 3.0 ml methylene chloride. The solution is added to 27.0 ml of human serum abumin solution (1% w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000–18,000 psi while recycling the emulsion for at least 5 cycles. The resulting system is transferred into a Rotary evaporator, and methylene chloride is rapidly removed at 40° C., at reduced pressure (30 mm Hg), for 20–30 minutes. The resulting dispersion is translucent, and the typical diameter of the resulting paclitaxel particles was 120–140 nm (Z-average, Malvern Zetasizer). The dispersion was filtered through a 0.22 micron filter (Millipore).

The sterile dispersion was further lyophilized for 48 hrs. without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

EXAMPLE 10

Extremely Small particle formation with a model drug

The effect of ethanol addition on reducing particle size is demonstrated for Isoreserpine. Thus, 30 mg Isoreserpine is dissolved in 2.7 ml methylene chloride and 0.3 ml ethanol. The solution is added to 27.0 ml of human serum abumin solution (1% w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification was performed at 9000–18,000 psi while recycling the emulsion for at least 5 cycles. The resulting system was transferred into a Rotary evaporator, and methylene chloride was rapidly removed at 40° C., at reduced pressure (30 mm Hg), for 20–30 minutes. The resulting dispersion was translucent, and the typical diameter of the resulting paclitaxel particles was 90–110 nm (Z-average, Malvern Zetasizer). The dispersion was filtered through a 0.22 micron filter (Millipore).

The sterile dispersion was further lyophilized for 48 hrs. without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

EXAMPLE 11

Use of a Water miscible Solvent alone, supersaturated with drug—Not suitable for invention process

30 mg Taxol is dispersed in 0.6 ml ethanol. At this concentration (50 mg/ml), the taxol is not completely soluble and forms a supersaturated dispersion. The dispersion is added to 29.4 ml of human serum abumin solution (1% w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude dispersion, and then transferred into a high pressure homogenizer (Avestin) The emulsification is performed at 9000–18,000 psi while recycling the emulsion for at least 6 cycles. The resulting system is transferred into a Rotary evaporator, and the ethanol is rapidly removed at 40° C., at reduced pressure (30 mm Hg), for 15–30 minutes. The resulting dispersion particle size is extremely broad, ranging from about 250 nm to several microns.

Observation under the microscope revealed the presence of large particles and typical needle shaped crystals of taxol.

These particles were too large for intravenous injection. This experiment demonstrates that the use of solvents such as ethanol that are freely miscible in water in the invention process results in the formation of large particles with very broad particle size distribution and as such cannot be used alone for the invention process. Thus the invention process specifically excludes the use of water miscible solvents when used alone for the dissolution or dispersion of the drug component. The invention process requires that such solvents, when used, must be mixed with essentially water immiscible solvents to allow production of the invention nanoparticles.

EXAMPLE 12

Use of a Water miscible Solvent alone containing dissolved drug—Not suitable for invention process

30 mg Taxol is dispersed in 1.3 ml ethanol. At this concentration (approx. 24.5 mg/ml), the taxol is completely soluble in ethanol. The solution is added to 28.7 ml of human serum abumin solution (1% w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I. Q.) in order to form a crude dispersion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000–18,000 psi while recycling the emulsion for at least 6 cycles. The resulting system is transferred into a Rotary evaporator, and the ethanol is rapidly removed at 40° C., at reduced pressure (30 mm Hg), for 15–30 minutes. The resulting dispersion particle size was extremely broad, ranging from about 250 nm to several microns. Observation under the microscope revealed the presence of large particles and typical needle shaped crystals of taxol. These particles were too large for intravenous injection.

This example, in addition to Example 11 above, demonstrates that the use in the invention process of solvents such as ethanol that are freely miscible in water results in the formation of large particles with very broad particle size distribution and as such cannot be used alone for the invention process. Thus the invention process specifically excludes the use of water miscible solvents when used alone for the dissolution or dispersion of the drug component. The invention process requires that such solvents, when used, be mixed with essentially water immiscible solvents to enable formation of invention nanoparticles.

EXAMPLE 13

Determination of Physical State of Paclitaxel in Nanoparticle Form by X-Ray Powder Diffraction

Paclitaxel raw material is usually present as needle shaped crystald of varying sizes typically between 5–500 microns. The presence of crystals in a drug formulation for intravenous injection is obviously detrimental if crystals are present in size above a few microns due to potential blockage of capillaries. In addition, the solubility of drug crystals in general would be lower than for amorphous drug, thereby lowering the bioavailability of the drug following intravenous administration. It is also known that as the loading of the drug in a formulation is increased, the tendency for crystallization also increases. Thus it is advantageous that the formulation contain the drug in essentially amorphous form.

X-Ray powder diffraction was used to determine the crystalline or non-crystalline nature of paclitaxel in the lyophilized powder formulation. The following samples were analyzed: Sample 1—Paclitaxel powder; Sample 2—Lyophilized serum albumin; Sample 3—a physical mixture of paclitaxel and albumin; and Sample 4—formulated paclitaxel. Each sample was x-rayed from 2° to 70° 2θ angles using CuKα radiation, an accelerating voltage of 40 KeV/30 mA, a step size of 0.050° 2θ and a data acquisition time of 2.0 seconds per step. Sample 1 showed strong peaks typical of a crystalline sample. The most intense paclitaxel peak was located at 5.10° 2θ. Sample 2 showed broad humps typical of amorphous material. Sample 3 showed largely the broad humps of Sample 2, but in addition, the peak at 5.10° 2θ of paclitaxel was visible. Sample 4, the formulated paclitaxel showed no evidence of crystallinity characteristic of paclitaxel and appeared identical to Sample 2, indicating the presence of substantially amorhpous pharmacologically active agent in the formulated sample.

The amorphous nature of the nanoparticles produced according to the invention stands in direct contrast to the products produced by other methods described in the art for producing nanoparticles. For example, the use of grinding techniques, as described in U.S. Pat. No. 5,145,684 (Liversidge et al.), and as described by Liversidge-Merisko et al., *Pharmaceutical Research* 13 (2):272–278 (1996), produces a substantially crystalline product.

EXAMPLE 14

Treatment of Tumors in an Animal Model with Paclitaxel Nanoparticles

Nanoparticles of paclitaxel (taxol) were prepared as described above in Example 1. This formulation of the drug was tested in a MX-1 human mammary tumor xenograft model in mice. The mice were implanted subcutaneously with the MX-1 mammary tumor and the treatment was initiated when the tumor reached approximately 150–300 mg in size. This occurred by day 12 and the treatment was initiated on day 13 after initial seeding.

Tumor bearing mice were treated with paclitaxel nanoparticles at a dose of 20 mg/kg, given by bolus intravenous injection as a suspension in saline for five consecutive days. The treated group included five animals. The control tumor bearing group of five animals received only saline on the same schedule. The size of the tumors was monitored as a function of time. The control group showed a tremendous increase in tumor weight. All the animals in this group were sacrificed between day 28 and day 39. The treatment group on the other hand showed remarkable efficacy as all animals had no measurable tumors by day 25. The animals in this group were all sacrificed on day 39, at which time they showed no evidence of recurrence and no evidence of tumor. The results are shown in FIG. 1.

EXAMPLE 15

Treatment of Rheumatoid Arthritis in an Animal Model with Paclitaxel Nanoparticles

Collagen induced arthritis model in the Louvain rat was used to test the therapeutic effect of Paclitaxel nanoparticles on arthritis. The paw sizes of the experimental animals were monitored to evaluate the seriousness of arthritis.

After the arthritis was fully developed (usually ~9–10 days after collagen injection), the experimental animals were divided into different groups to receive either Paclitaxel nanoparticles 1 mg/kg q.o.d, or Paclitaxel nanoparticles 0.5 mg/kg+Prednisone 0.2 mg/kg q.o.d. (combination treatment) intraperitoneally for 6 doses, then one dose per

week for three weeks. The paw sizes were measured at the beginning of treatment (day 0) and every time the drug was injected. One group received only normal saline as control. By the end of the experiment, the group receiving Paclitaxel nanoparticles achieved a 42% reduction of paw size, the combination treatment group showed a 33% reduction of the paw size while the control group had about 20% increase of the paw size. Original paw size before arthritis was induced was 50%. The results are shown in FIG. 2.

In conclusion, the paclitaxel-containing nanoparticles demonstrated therapeutic effect on arthritis. To avoid side effects of long term use of both paclitaxel and the steroid, it is probably better to choose a combination treatment to get similar effect but only half the dosage of each drug.

EXAMPLE 16

In vivo Targeting of Nanoparticles

By incorporation of certain targeting moieties such as proteins, antibodies, enzymes, peptides, oligonucleotides, sugars, polysaccharides, and the like, into the protein coating of the nanoparticles, it is possible to target specific sites in the body. This targeting ability can be utilized for therapeutic or diagnostic purposes.

EXAMPLE 17

Intravenous Delivery Systems Formulated From a Variety of Materials

The materials used for the preparation of intravenous delivery systems may be polymeric (e.g., polyethylene, polyvinyl, polypropylene tubing, and the like), or glass. Standard medical grade tubing is known to contain hydrophobic moieties on the inner surfaces thereof. These moieties are thus available to come in contact with the injection solution. Indeed, such tubing is specifically tailored, as are the catheters, to present hydrophobic moieties in contact with the treatment solution so as to reduce the absorption of aqueous material to the tubing. However, any hydrophobic moieties in the treatment solution will likely bind to both the catheter tubing and other components of the delivery system. As a result, a substantial portion of a hydrophobic pharmacalogically active agent can become sequestered in the inner walls of the tubing catheter and delivery vessel. Consequently, the dosing of hydrophobic pharmacologically active agents can be erratic, since a substantial portion of the active agent can become absorbed to the walls of the tubing. In critical therapeutic treatments, where the hydrophobic pharmacologically active agent is used to treat a disease, a significant reduction in the effective dose of active agent can lead to a therapeutic failure. The failure is particularly striking when employing therapeutic moieties which require that the active agent be present above a certain level, yet the therapeutic window is narrow.

A novel method for the intravenous introduction of a hydrophobic pharmacologically active agent has now been developed. By protecting the hydrophobic moieties of the active agent, through association with the hydrophobic moieties of a biocompatible coating (e.g., albumin), the propensity of the active agent to become attached to the tubing is dramatically reduced. Thus, the present invention enables the use of highly hydrophobic drugs, in combination with standard medical grade polymers and hydrophobic glasses, in which the drug is protected and therefore not absorbed onto the surface. The invention method comprises placing a protective coating of a biocompatible polymer (e.g., albumin) around the hydrophobic drug and placing the resulting composition in a hydrophobic polymeric delivery system. The invention methods are therefore capable of improving the delivery of a variety of hydrophobic therapeutics.

EXAMPLE 18

Intravenous Administration of Therapeutics

Intravenous administration of therapeutics, for example, drugs, imaging agents, and the like, predisposes the therapeutic to at least one pass through the liver. As that therapeutic is filtered through the liver, a significant portion of that therapeutic is taken up and sequestered by the liver, and therefore, not available for systemic distribution. Moreover, once taken up by the liver, it is likely to be metabolized, and the resulting metabolic byproducts often have general systemic toxicities. By encapsulating the drug or other therapeutic agent in a coating according to the invention (e.g., using a protein such as albumin), liver sequestration upon intravenous administration is alleviated. Albumin, for example, is known to pass through the liver and become generally distributed throughout the patient. Thus, the sequestration of albumin by the liver does not occur to the same degree as toxic compounds or drugs which have hepatic receptors (or other mechanisms) which initiate processes which result in their removal from the blood stream. By protecting the therapeutic with a coating of a biocompatible polymer (e.g., a human albumin coating), the drug then bypasses the liver and is generally distributed through all organ systems. In accordance with one aspect of the present invention, there is provided a novel method for bypassing the liver, which comprises encapsulating a drug in a human liver albumin (essentially a physiological component). In this way, more of the drug becomes available for systemic therapy. In addition to the increased availability of the drug, there is a decrease in the production of metabolic byproducts of hepatocellular drug degradation. Both the increase in liver bypass and decrease in byproducts of drug metabolism provide a synergistic improvement in the overall drug efficacy. This improved efficacy extends to all drugs and materials that are encapsulated in human albumin.

EXAMPLE 19

Reducing Myelosuppressive Effects and General Toxicity of Drugs

Several chemotherapeutic drugs have dose limiting toxicity due to their myelosuppressive effects. Taxol (paclitaxel) is a classic example of such a drug. When administered in its currently approved formulation of cremaphor/ethanol, taxol produces myelosuppressive effects that limit the repeat administration of the drug and preclude retreatment of a patient for at least 3 weeks in order to allow blood counts of the patient to return to normal. It was postulated that due to the non-toxic compatible nature of the drug carrier of the present invention, viz. human albumin, the toxic side effect of myelosuppression may be greatly reduced.

Sprague dawley rats were given paclitaxel in commercial formulation (available from Bristol Myers Squibb (BMS) in cremaphor/ethanol) or prepared by the invention method as nanoparticles with albumin. Both formulations were administered by tail vein injection. A single dose level of 5 mg/kg was administered for the BMS formulation, whereas two dose levels of 5 mg/kg and 12 mg/kg were administered for

the invention formulation (Capxol). The white blood cell counts of the rats were monitored daily after administration as an index of myelosuppression.

For the BMS formulation (5 mg/kg) it was found that the WBC counts dropped by 47.6% and 63.5% on day 1 and day 2 after administration, respectively, whereas for the Capxol formulation at 5 mg/kg, the WBC counts increased by 14.7% and 2.4% on day 1 and day 2, respectively. For the higher dose Capxol at 12 mg/kg, the WBC counts increased by 6.5% and 3.6% on day 1 and day 2, respectively.

These results indicate that short term myelosuppression is greatly reduced by administering the drug in the present invention formulation.

Another indicator of general toxicity is the body weight of the animal. Body weights of the rats were also monitored following administration of paclitaxel. At a dose of 5 mg/kg, the BMS formulation resulted in a reduction of body weight by 10.4% in 3 days following administration, whereas the same dose of paclitaxel administered in the invention formulation (Capxol) resulted in only a 3.9% drop in body weight, indicating the greatly reduced toxicity of the invention formulation.

EXAMPLE 20

Administration of Bolus dose of Nanoparticle Formulation

The anticancer drug, paclitaxel, in its commercial BMS formulation with Cremaphor/ethanol, cannot be administered as an intravenous bolus. This is due to the extensive toxicity of the vehicle which results in severe anaphylactic reactions and requires patients receiving the drug to be pre-medicated with steroids, antihistamines, and the like. The BMS formulation is administered as an intravenous infusion lasting anywhere from 1 hour to 24 hours. In contrast, formulations according to the present invention, due to the use of a non-toxic carrier, can be administered to a patient readily as an intravenous bolus (i.e., in a period less than 1 hour) without the toxicity problems seen in the BMS formulation that is used clinically today.

The effective dose of paclitaxel for a patient typically lies between 200–500 mg, depending on the patient body weight or body surface. The BMS formulation has to be administered at a final dosing concentration of 0.6 mg/ml, requiring large infusion volumes (typically in the range of about 300–1000 ml. In contrast, invention formulations (e.g., Capxol) do not have these limitations and can be administered at a desired concentration. This enables clinicians to treat patients by a rapid intravenous bolus that can be administered in as little as a few minutes. For example, if the invention formulation is reconstituted to a dosing concentration of 20 mg/ml, the infusion volume for a total dose of 200–500 mg is only 10–25 ml, respectively. This is a great advantage in clinical practice.

EXAMPLE 21

Reduction in Toxicity of Paclitaxel in the Nanoparticle Formulation Compared to the Commercial Cremaphor/Ethanol Formulation

It is well known that the anticancer drug, paclitaxel, in its commercial BMS formulation with Cremaphor/ethanol, has extensive toxicity which results in severe anaphylactic reactions and requires patients receiving the drug to be pre-medicated with steroids, antihistamines, and the like. The toxicity of the BMS formulation was compared to the nanoparticle formulation of the present invention.

Thus, the formulations were injected intravenously through the tail vein of C57BL mice at different dose levels and toxic effects were monitored by general observation of mice after the injection.

For the BMS formulation, a dose of 30 mg/kg was uniformly lethal within 5 minutes of intravenous administration. For the same dose, the nanoparticle formulation according to the invention showed no apparent toxic effects. The nanoparticle formulation at a dose of 103 mg/kg showed some reduction in body weight of the mice, but even this high dose was not lethal. Doses of approximately 1000 mg/kg, 800 mg/kg and 550 mg/kg were all lethal but differing in time to lethality, which ranged between a few hours to 24 hours. The lethal dose of the invention formulation is greater than 103 mg/kg but less than 550 mg/kg.

Thus, the lethal dose of the invention formulation of paclitaxel is substantially higher than that of the commercial BMS formulation. This has great significance in clinical practice where higher doses of chemotherapeutic drugs may be administered for more effective oncolytic activity with greatly reduced toxicity.

EXAMPLE 22

Preparation of Nanoparticles of Cyclosporine (Capsorine I.V.) by High Pressure Homogenization

30 mg cyclosporine is dissolved in 3.0 ml methylene chloride. The solution is then added into 27.0 ml of human serum albumin solution (1% w/v). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer model: Tempest I.Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification was performed at 9000–18,000 psi while recycling the emulsion for at least 5 cycles. The resulting system was transferred into a Rotavap and methylene chloride was rapidly removed at 40° C., at reduced pressure (30 mm Hg), for 20–30 minutes. The resulting dispersion was translucent and the typical diameter of the resulting cyclosporine particles was 160–220 (Z-average, Malvern Zetasizer).

The dispersion was further lyophilized for 48 hours, without adding any cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline. The particle size after reconstitution was the same as before lyophilization.

EXAMPLE 23

Preparation of Nanodroplets of Cyclosporine (Capsorine Oral) by High Pressure Homogenization

30 mg cyclosporine is dissolved in 3.0 ml of a suitable oil (sesame oil containing 10% orange oil). The solution is then added into 27.0 ml of human serum albumin solution (1% v/w). The mixture is homogenized for 5 minutes at low RPM (Vitris homogenizer, model: Tempest I.Q.) in order to form a crude emulsion, and then transferred into a high pressure homogenizer (Avestin). The emulsification is performed at 9000–18,000 psi while recycling the emulsion for at least 5 cycles. The resulting dispersion had a typical diameter of 160–220 (Z-average, Malvern Zetasizer).

The dispersion could be used directly or lyophilized for 48 hours by optionally adding a suitable cryoprotectant. The resulting cake could be easily reconstituted to the original dispersion by addition of sterile water or saline.

EXAMPLE 24

Pharmacokinetic (PK) Data for Cyclosporine Nanoparticles (Capsorine I.V.) Following Intravenous Administration Comparison with Sandimmune I.V. (Currently Marketed Formulation by Sandoz)

Nanoparticles of cyclosporine (Capsorine I.V.) prepared as described above (Examples 22 and 23) were reconstituted

in saline and administered to a first group of 3 Sprague Dawley rats by intravenous bolus. A second group of 3 rats were given Sandimmune I.V., which contains Cremaphor/Ethanol after dilution in saline. Each group received the same dose of 2.5 mg/kg. Blood samples were taken at times 0, 5, 15, 30 (minutes) 1, 2, 4, 8, 24, 36 and 48 (hours). Levels of cyclosporine in the blood were assayed by HPLC and typical PK parameters were determined. The PK curves showed typical decay over time as follows:

	Decay Over Time	
	AUC, mg-hr/ml	Cmax, ng/ml
Capsorine I.V.	12,228	2,853
Sandimmune I.V.	7,791	2,606

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

EXAMPLE 25

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

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

	Decay Over Time	
	AUC, mg-hr/ml	Cmax, ng/ml
Capsorine Oral	3,195	887
Neoral	3,213	690

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

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

That which is claimed is:

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

subjecting a mixture comprising:

an organic phase containing said pharmacologically active agent dispersed therein, and

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

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

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

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

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

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

7. A method according to claim 4 wherein said pharmaceutically active agent is an immunosuppressive agent selected from cyclosporine, azathioprine, mizoribine or FK506 (tacrolimus).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

subjecting a mixture comprising:

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

aqueous medium containing biocompatible polymer,

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

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

28. A method according to claim 26 further comprising filtering said mixture through a 0.22 micron filter.

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

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

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

* * * * *

IN THE UNITED STATES DISTRICT COURT

IN AND FOR THE DISTRICT OF DELAWARE

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ELAN PHARMA INTERNATIONAL LIMITED,	:	Civil Action
Plaintiff,	:	
v.	:	
ABRAXIS BIOSCIENCE INC.,	:	
Defendant.	:	No. 06-438-GMS

- - -

Wilmington, Delaware
Friday, June 6, 2008
8:45 a.m.
FIFTH DAY OF TRIAL

- - -

BEFORE: HONORABLE GREGORY M. SLEET, Chief Judge, and a Jury

APPEARANCES:

JOHN G. DAY, ESQ.
Ashby & Geddes
-and-
STEPHEN SCHEVE, ESQ.,
LINDA M. GLOVER, ESQ.,
JEFFREY SULLIVAN, ESQ.,
ROBERT RIDDLE, ESQ., and
PAUL FEHLNER, ESQ.
Baker Botts LLP
(Houston, TX)
-and-
GREGORY BOKAR, ESQ.
Counsel - Elan Drug Delivery

Counsel for Plaintiff

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editorial capacity?
A. Yes. I have done a lot of editorial work over the years. In fact, we are pulling it up here, I have edited several journals and actually founded three journals over the years.
Q. What journals did you found, sir?
A. I founded a journal called the Journal of Inclusion Phenomena in 1983. I then founded a journal called Supramolecular Chemistry in 1990. And I founded The Journal of Supramolecular Chemistry in about 2002.
Q. Back to peer-reviewing, do you serve as a reviewer on peer-reviewed journals?
A. Yes. I review probably a paper a week on peer-reviewed journals. In addition to my editorial job which is to evaluate work that comes in from the outside to journals with which I am associated.
Q. What is your task when you are doing the peer-review function?
A. The peer-review function is to look at the science that is presented, and to make a decision: Is this good science? Is this based on solid information? Is the data appropriate for the conclusions that are being drawn?
Q. Have you received any awards for your work, sir?
A. Yes, I have.
Q. Can you just identify a few of those?

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A. The award that's the most meaningful to me is the Izatt Christiansen International Macrocyclic Award in the year 2000. This is a special area of chemistry that is important to supramolecular chemistry.
Others that I am particularly proud of, I have had a long association with chemists in Poland. I received the honorary medal of the Institute of Physical Chemistry three years ago. I was elected the foreign member of the Polish Academy of Sciences sometime before that.
Q. Could we have DX-611 on the screen, please? 611.
What is DX-611, Dr. Atwood?
A. It has quite a nice picture on it.
But other than that, I was surprised, just this past month, to learn that some of my colleagues from around the world had put together a special issue honoring me, surprised, and, unfortunately, it was on the occasion of my 65th birthday.
Q. Do you have experience in determining crystallinity and amorphousness in substances?
A. Yes, I do. I have considerable experience in that area.
Q. Can you describe that a little bit?
A. The gold standard, as we have heard in this litigation, for determining crystallinity is x-ray powder diffraction. I did my first x-ray powder pattern 45 years

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ago this month. I personally did my most recent x-ray powder pattern a week ago yesterday.
Q. How about other analytical techniques for determining crystallinity or amorphousness, do you have experience with them?
A. I do. As I said, x-ray powder diffraction is the gold standard. But there are certain other supportive techniques, electron microscopy, solid-state NMR, and as one determining crystallinity, one may draw inferences from other techniques.
But the nice thing about x-ray powder diffraction is it's really quite definitive for determining amorphousness or crystallinity.
Q. Dr. Atwood, before this, your involvement in this lawsuit, did you have any relationship with Abraxis or its principal executives?
A. No, I did not.
Q. How about a relationship with Elan, did you have any previous relationship with them?
A. No. I have had no relationship with either Elan or Abraxis prior to this litigation.
Q. Are you an inventor on any patents, Dr. Atwood?
A. Yes, I am.
Q. Could you describe your involvement in the patent system?

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A. I have ten patents that have been issued so far. My initial patents -- most of my patents are related to trying to solve the energy problem and using supramolecular techniques to try to break our dependence on foreign oil.
I have three patents related to deriving fuel from coal from quite a number of years ago. And more recently, my patents have been with regard to fuels related to how one can store hydrogen or natural gas for propelling automobiles.
I have a few patents, also, on my other true love. That is nanocapsules, particularly nanocapsules related to targeted drug delivery.
Q. Can you just tell us a minute about nanocapsules and your work with them. How did that develop?
A. I have had a long-term interest in what we used to call inclusion chemistry. Inclusion chemistry is, like my hand is the molecule, it could include something like this, but it could also release it.
This area eventually, over the years, developed into a branch of supramolecular chemistry. And in supramolecular chemistry, we are able to put together capsules of anywhere from three or four nanometers diameter up to about 80 nanometers in diameter. And we are able to include into these capsules not just like the pointer into the hand but to really include within the capsules drug

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this mathematics of needing three peaks but only finding one so we will double the one peak we have to get us to eight percent, this is the kind of thing that I wish the bank would do for me occasionally, double something that's not there into something substantial.
Q. Did Dr. Munson identify an error rate with the analysis that he performed?
A. He did.
Q. What was that error rate?
A. The error rate was 50 percent, which, in scientific terms, is huge.
Q. So, Dr. Atwood, let's just review here what you did versus what Dr. Munson did with the ssNMR analysis.
You did what kind of analysis of the crystalline control, the manipulated Abraxane, and the amorphous, to reach your conclusion as to what ssNMR illustrated about Abraxane?
A. The analysis I did was really very simple. The center pattern is the manipulated Abraxane. This is the important pattern. And I asked myself, Does this look like amorphous or does it look crystalline?
Well, it's clear it looks amorphous. But I then superimposed the two, just to see if it just looked that way or if it really was virtually identical. And it was a great match.

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Q. Then Dr. Munson -- sorry. Did Dr. Munson do that analysis?
A. Dr. Munson did not do that analysis.
Q. And Dr. Munson went on to do deconvolution, and you analyzed his deconvolution, and what was your conclusion?
A. My conclusion from his deconvolution is, there is no third peak.
Q. And if there is no third peak, Dr. Atwood, what happens to Dr. Munson's conclusion that there is evidence of crystallinity in Abraxane?
A. Then there is no evidence of crystallinity in Abraxane from the solid state NMR. That third peak was Dr. Munson's key.
But, remember, I have just been pointing out that that third peak really doesn't get it, because we don't need one additional peak, we need at least two and probably three additional peaks. And we need them in these positions.
The little peak which Dr. Munson placed in didn't even match the little one here. It was placed about there. So it completely missed the big peak and the second big peak.
Q. Dr. Atwood, putting all of the evidence that you have looked at together, the x-ray powder diffraction, the ssNMR, what is your conclusion as to whether the paclitaxel in the

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nanoparticles in Abraxane is crystalline or amorphous?
A. It's quite clear the paclitaxel in Abraxane is amorphous.
Q. Just walk us through one more time what you are relying on.
A. I am basing that on the 13 XRPD tests at the top, and I am substantiating that with Dr. Munson's solid state NMR work.
MR. JACOBS: Thank you very much, Dr. Atwood.
THE COURT: Thank you, Mr. Jacobs. I think what we will do is take our lunch early today. That way cross won't have to be broken up, which I suspect it might if we were to start now.
Ladies and gentlemen, let's take our hour right now.
(Jury leaves courtroom at 12:33.)
(Luncheon recess taken.)
MR. DAY: A couple of housekeeping matters, Your Honor?
On Tuesday afternoon, in the conversation in which Your Honor mentioned that the case was going to be going to the jury on Tuesday, you also told us you wanted jury instructions done by today.
THE COURT: At least in shape to have a meaningful conversation about them.

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MR. DAY: We have made significant progress on that. We are hopeful we might be able to get those to you on Monday morning rather than today.
THE COURT: That is fine.
MR. DAY: Terrific. Second one, Your Honor. I am planning to be here at 8:30 for the Aventis v. Barr closing argument on Monday morning, as I know you are. I suspect Ms. Walker is. I also expect that means we won't be starting at 9:00 on Monday morning here.
THE COURT: That's right. I am glad you reminded me of that.
MR. DAY: Thank you, Your Honor.
MR. JACOBS: A question, Your Honor, of your procedure. Mr. Scheve has kindly alerted me that he intends to try to impeach Dr. Atwood from testimony in other lawsuits in which Dr. Atwood testified. I don't know what it is. We haven't seen it before.
What is your procedure, Your Honor, for impeaching, No. 1, with material which hasn't been disclosed to us before, and No. 2, with material from other lawsuits in other contexts.
THE COURT: Mr. Scheve, what is your proposal to the Court as to, No. 1, how you want to do that and why you should be able to do it?
MR. SCHEVE: It is impeaching, Your Honor. I

IN THE UNITED STATES DISTRICT COURT

IN AND FOR THE DISTRICT OF DELAWARE

- - -

ELAN PHARMA INTERNATIONAL LIMITED,	:	Civil Action
Plaintiff,	:	
v.	:	
ABRAXIS BIOSCIENCE INC.,	:	
Defendant.	:	No. 06-438-GMS

- - -

Wilmington, Delaware
Monday, June 9, 2008
8:30 a.m.
SIXTH DAY OF TRIAL

- - -

BEFORE: HONORABLE GREGORY M. SLEET, Chief Judge, and a Jury

APPEARANCES:

JOHN G. DAY, ESQ.
Ashby & Geddes
-and-
STEPHEN SCHEVE, ESQ.,
LINDA M. GLOVER, ESQ.,
JEFFREY SULLIVAN, ESQ.,
LISA A. CHIARINI, ESQ.
ROBERT RIDDLE, ESQ., and
PAUL FEHLNER, ESQ.
Baker Botts LLP
(Houston, TX)
-and-
GREGORY BOKAR, ESQ.
Counsel - Elan Drug Delivery

Counsel for Plaintiff

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blood and into the tumor in a very quick fashion. Therefore, prevent toxicities, but maybe even help fight the tumor.

So that was, really, how it all began.

Q. When did you actually begin working on a paclitaxel drug delivery system?

A. That would have been, I think, 1992, to the best I remember.

Q. Let's go to the other end of the chronology and talk about Abraxane and what's actually in an Abraxane vial.

What does Abraxis place in a vial of Abraxane?

A. Well, Abraxane is really composed of only two components. It has a lot of albumin, which is the protein that I just talked about, and it has the drug paclitaxel.

The drug paclitaxel, or Taxol, as it is known as, is in the form of these very tiny particles, which are amorphous in nature, that is, they are not crystalline. And these are nanometer sized particles. To be specific, they are about 130 nanometers.

Just to calibrate you, I am not sure if you are familiar with nanometer size range, but it's about, if you think of the thickness of a human hair, and that is a common example that everybody uses, and you think of the size of the nanoparticle, it is about 10,000 times smaller than the thickness of the human hair.

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Q. So, in sum, what is in the vial?

A. In the vial, there is about ten times as much albumin as there is the drug paclitaxel, so it's a ratio of roughly nine or ten to one. And the paclitaxel is in the form of these amorphous nanoparticles. And around the nanoparticles is a thin layer of the albumin, which is cross-linked.

Q. What is Abraxane approved for today?

A. Today it is approved for the treatment of breast cancer.

Q. What other areas are being explored?

A. The other types of cancers that have been tested for, and are in clinical trials this very moment, are in lung cancer, in melanoma, in ovarian cancer, people have interest in brain cancer, and, right now, we are getting some very exciting results in pancreatic cancer.

Q. Breast cancer was the first disease for which it was improved?

A. That's correct, yes.

Q. Why did you, at Abraxis, pursue breast cancer first?

A. Breast cancer, as you know, like all other cancers, is a terrible disease. It affects a large population of women. And we thought this might be a good place to test the drug, since there is a big need for new drugs in breast cancer.

The drug Taxol, which was approved in 1993, was a big advance in breast cancer, but it had lots of

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limitations. One of the big limitations was the toxicity of that drug, and toxicity was really not due to the active drug itself, but due to what was a solvent that was given with the drug, just to help make it soluble, so that it could be injected into the bloodstream of cancer patients.

And patients actually died from receiving that, because of the toxicity of the solvent.

So there was a big need at that point. And we decided that using our approach of albumin to get the drug out of the bloodstream very fast to the tumor would be appropriate in this setting, so that we could make it safe and we could also make it more effective.

Q. You mentioned the size of the nanoparticles in the Abraxane formulation.

Why is it important that the size of those particles be what it is?

A. It's actually critical that the size be in the range, or well below 200 nanometers, because when you think about drugs that are administered directly into the bloodstream, you have to be -- it's a very high standard of safety you need to achieve because you cannot have any contaminants. You cannot have any microorganisms. You cannot have bugs accidentally in the preparation, because since the drug goes directly into the bloodstream, you can have very adverse events for the patient.

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So the size is very important, because the FDA has demanded that for a drug to be injected in the bloodstream, it needs to be sterile, that is, it cannot have any bacterial contamination. The way to avoid that is to pass the drug through tiny pore filters which have very tiny pores, which are 200 nanometers. Since most microorganisms are bigger than 200 nanometers, you would filter them out. This drug needs to be put through these filters. Therefore, if the size is greater than 200 nanometers, it is not getting through the filter.

You have to have it sufficiently smaller than 200 nanometers, about 150, 130 nanometers, so you can pass the entire drug through, thereby sterilize it and have it safe. That is why the size is very important.

Q. Can you tell the jury why Abraxane was designed to be the way you described it, amorphous, cross-linked, and maybe any other reasons why you designed it to be nano-sized?

A. So the amorphous nature has really got to do with how fast you can get the drug to the tumor. When you have something amorphous, that is, amorphous really means that you have a lack of structure, and when you have that kind of molecule as opposed to a crystalline molecule, which is the opposite of amorphous, amorphous molecules dissolve very fast. It's like taking the analogy of cotton candy. You put cotton candy on your tongue, it just goes away and

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dissolves right away, versus the rock candy example. These are big crystals of rock, of candy that take a long time to dissolve.

So using that analogy, we thought of amorphous as being very useful for dissolving the drug very fast, and because we use albumin, it helps to get the drug right out of the bloodstream like that, very quickly.

The second aspect to it is the cross-linking aspect. We are creating these structures, which are small spherical balls, if you will, of about 130 nanometers in size. We need something to keep that structure in place as we produce through our manufacturing process.

The idea of having albumin as a cross-linked shell around it helps to stabilize that structure. And by "cross-linked," I mean it's like a mesh or a net so the individual albumin molecules are linked together to create this net or mesh that holds the structure together.

Those are the critical features, sort of, of Abraxane.

Q. Any other reasons besides sterility why you made it so small?

A. Yes. Other than the sterility aspect, it was well-known at the time that if you had small particles in the hundred nanometer size range, this is important for getting out of the bloodstream into the tumor, as opposed to

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larger particles, which may go into other organs, like the liver and spleen. We did not want these particles to get into these other organs, but we wanted to get them into the tumor.

Q. Over time, have you developed your own understanding of Elan's approach to nano-sized particles?

A. Yes. Certainly over time, through presentations that I have heard and through other materials, we have come to understand Elan's technology as being very interesting and novel, but focused on a different aspect. They focus on crystals. And their technology focuses on breaking down crystals to small size. So you start from a big crystal and end up with a smaller crystal in the nano-size range.

As compared to that, our technology is different, we actually build the nanoparticle to make an amorphous nanoparticle.

The other thing I learned about Elan's technology is that they put surface modifiers on the surface of the crystal, and that these surface modifiers, it turns out, are non-cross-link. They are not linked to each other. The individual molecules of the surface modifier are separate, which is again different from the approach we used, that our surface modifiers of proteins is a protein albumin, and is linked together to form this shell around the particle.

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Q. Let's use a slide to explain this to the jury, Dr. Desai. Does this reflect your understanding of the differences between Elan's technology, as you came to learn it, and Abraxis' technology?

A. That's correct. It does. On the left side is Elan's technology, and on top left first, so these are crystals of the drug paclitaxel. And the paclitaxel crystals, a very characteristic feature of paclitaxel, they form needle-like or rod-like crystals. That is why this shape.

And then on the, those crystals are adsorbed, which means just stuck there without any chemical interaction, these molecules of a surface modifier that are non-cross-linked, that is, they are not attached to each other in any way.

So that forms the basis of Elan's technology as it relates to paclitaxel.

On the other hand, Abraxane, as I tried to describe to you, is more, sort of a spherical structure. It's like a little ball of paclitaxel where the drug, itself, in the core of the particle is amorphous. So we don't have crystals. And as I mentioned, crystals of paclitaxel look like this, like little rods.

On the surface of the particles, this is designed to have albumin, the protein albumin on the surface that is cross-linked to each other. That is the individual

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molecules of albumin are linked to each other to stabilize this particle.

Q. How long have you been working with paclitaxel?

A. I have been working with paclitaxel from about 1992. That would probably make it, what, 16 years or so.

Q. Have you ever seen it in a form different than the form you are describing it here, that is, needle shaped or rod shaped?

A. In my entire career working with paclitaxel, any time you look at paclitaxel crystalline material that is from many different manufacturers that we have looked at, it's always in the form of needle-shaped crystals. In fact, in the textbooks, it's even described as needle-shaped crystals for paclitaxel. That is a very characteristic feature.

Q. Did you develop an understanding of how Elan manufactures its nanoparticles?

A. Well, from the information that was out in the literature, it was evident that Elan uses some techniques where we start with big crystals, which may have come from a manufacturer, from a manufacturer of this drug, and these crystals are then ground up into small particles.

One of the ways of grinding that I think Elan uses is using what's called a ball mill. In essence --

MR. SCHEVE: Excuse me, Your Honor. To the extent he just expressed what he thinks Elan does, there is

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no foundation.
BY MR. JACOBS:
Q. Can you explain how you came to have the understanding about Elan's technology reflected on this slide?
A. Yes. From reading in the literature, from reading in published patents, and from some materials that they sent to us, I think, in 1996. And from presentations that I have heard at scientific meetings.
So at any rate, the technology, as I understand it, involves grinding of large crystals to make them into small crystals, and then, also, in the presence of a surface modifier to help stabilize these crystals.
On the other side is, just so you can get a comparison of the different process that we use for making Abraxane, here is how we make Abraxane. Again, this is simplified, so that we can easily understand it.
We have two components --
MR. SCHEVE: Your Honor, may we have a sidebar.
(The following took place at sidebar.)
MR. SCHEVE: This is clear comparison of the embodiment of the '363 to the Abraxane product. We object that that is inappropriate.
MR. JACOBS: I don't see how it can possibly be inappropriate for him for compare and contrast the manufacturing information when Mr. Scheve, in his opening

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statement, placed into question whether somehow Abraxis had copied manufacturing details from the information that Elan received in 1996.
THE COURT: Did you hear what he said?
MR. SCHEVE: I did. He is saying, Here is what Elan's product looked like, these needles. Now he is going to compare it to the accused product, the accused infringing product. That's clearly not appropriate.
MR. JACOBS: That is a different issue from manufacturing, which is what I think we are on right now.
MR. SCHEVE: He didn't put up a slide comparing manufacturing. He put up needles and he is going to compare it to Abraxane. That is inappropriate.
MR. JACOBS: I think the objection is, now he is about to explain the Abraxane manufacturing process. I think we are past the description of the Elan process. We are on to Abraxis now. We are about to move into a discussion of how Abraxane is made. I think it is very relevant to the jury.
THE COURT: He doesn't object to that.
MR. SCHEVE: No, not to how they make Abraxane. He has made a comparison, that is what I object to.
MR. JACOBS: He is describing his understanding, having worked in the field. They have put his state of mind at issue.

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THE COURT: You are finished now, on the manufacturing?
MR. JACOBS: Exactly.
MR. SCHEVE: As long as there is no comparison of their invention to their infringing --
THE COURT: Both of you well understand.
(End of sidebar conference.)
BY MR. JACOBS:
Q. Let's proceed with where we were. You were describing the manufacturing process for Abraxane by Abraxis.
A. On the right side here, we have the manufacturing process for Abraxane. It essentially starts with two components. One is albumin, the protein. This protein is dissolved in water. So you have a solution of albumin.
On the other hand, the other component is paclitaxel. So we start with large crystals of paclitaxel that we obtain from the paclitaxel manufacturer, not unlike what is shown here, the needle-shaped crystals. And we completely dissolve them in a solvent. So there are certain solvents that can dissolve paclitaxel so all the crystalline character is gone, it's completely dissolved. It's a solution. It's like taking sugar and putting it in water, and after some time, you can't see anything anymore. You can't see the particles.
So all of the crystalline character completely

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disappears. It's now entirely just molecules floating around in solution.
Now, the second step is to combine these two in a process, where you can disperse or make tiny droplets of this paclitaxel solution inside the albumin solution.
This is shown here as the little red dots. You make these tiny little droplets of the solution of paclitaxel inside this albumin solution. And then what you do is rapidly evaporate this solvent. So the idea is you only use this solvent initially to dissolve the material. But then you want to take it out. So you put it under a vacuum, and under vacuum, the solvent can evaporate off, or boil off, and leaving behind the drug.
What this does is, because it's in solution, and you take the solvent away, it actually precipitates the drug in an amorphous form. So when it comes out of the solution, you do what's known as a solvent precipitation, that is, the solvent goes away, and you precipitate or create solid particles off the paclitaxel, which is amorphous in character.
This is a well-recognized theme, that is, solvent precipitation can be used to create amorphous materials. So we used that theme.
After we evaporated the solvent, you end up with tiny solid particles, and here you have those solid

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amorphous particles. In that process here, the albumin settles around the surface of the nanoparticles and you get cross-linking.

So that's how you end up with these particles that are cross-linked and be amorphous.

Q. Have you prepared an animation to describe the Abraxis manufacturing process?

A. Yes. We can see that here.

Q. Why don't you explain to us what this animation is describing?

A. Okay. So this actually takes you through the process. As I mentioned, the first step, you take the paclitaxel crystals. You completely dissolve them in solvent. And the raw material you get for this is in the form of crystals. You put this into the solvent and stir it up and shake it up. Eventually, these crystals are completely gone. There is no more crystal structure. It is essentially a clear solution.

Now you have got the paclitaxel solution, which has no more crystals.

The second step to this is taking the albumin solution. Remember, there are two components, albumin and the drug, so here is the albumin dissolved in water, here is the paclitaxel dissolved in solvent, and we mix these up and we take the crystal-free paclitaxel solution and homogenize

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or mix it or fluidize it with the two together. The idea, as was seen in the previous picture, was to get these tiny droplets of the mixed solution.

So this is mixed up under high shear conditions so you get a very fine droplet size.

That will be seen in a second.

At this stage, you get the cross-linking of albumin. Around the droplets, the albumin accumulates. Then the cross-linking occurs. After this step is done, what we want to do, remember, the solvent is still here, we want to get rid of all the solvent. So if you look at this microscopically now, these are tiny droplets, as indicated here in the dark red, which contain the drug. And then these yellow particles, or these yellow molecules are actually the albumin that is beginning to accumulate on the surface and start cross-linking.

When you look at it close up, you can see that this is shown that there is individual molecules of paclitaxel that are still dissolved inside the droplet in the solvent. That's indicated here. And, again, there is no crystal structure here, remember. This is completely dissolved. And then on the surface of these particles is the albumin that starts to accumulate and coat the surface and cross-link to each other or link to each -- the molecules of albumin linked to each other.

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Now you have got these droplets and you do evaporation. You, in effect, under vacuum, you are removing all of the solvent. So the solvent goes away. And because of that, you precipitate, that is, you create these solid particles by solvent precipitation. These particles are amorphous.

Essentially, you have created, now you have cross-linked albumin coating on these solid particles, amorphous particles.

Now you have nanoparticles suspended in albumin solution. The next step is to filter. Remember I talked to you about the importance of filtration, to make sure that it become sterile. So it's filtered and then filled into the vials that we use for making Abraxane. At this point, you freeze dry it. That is, you freeze it rapidly, and you put it again under a vacuum, so all of the water evaporates off to leave you with a dry powder.

Here is the water coming off under high vacuum. And then all you are left with is a dry powder that has the two components, the albumin and the drug paclitaxel, and then the nanoparticles of paclitaxel, which are amorphous, and have a cross-linked coating.

Of course, we put a cap on the vial. And this is what becomes Abraxane. And then the 100 milligrams of drug in there, paclitaxel, with about ten times as much of

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the albumin.

Q. Dr. Desai, I am sure you recall, you had your deposition taken in this case. Do you remember that?

A. Yes, I do.

Q. Do you recall that Elan asked you a lot of questions suggesting that you had somehow used Elan's ideas from a packet of material that Gary Liversidge sent you around August 28th, 1996, to develop Abraxane. Did you do that?

A. Not at all. In fact, the approaches were completely different. By that time, we were well under way to developing Abraxane.

Q. I would like to run through with you a chronology of some of the milestones in the development of Abraxane before the exchange with Nano Systems in late August 1996.

This is a timeline, Dr. Desai. Did you help prepare this?

A. Yes, I did.

Q. Can you walk us through briefly what this timeline is showing?

A. Okay. So this timeline starts with early 1992, when we first think about and start to work on what eventually became Abraxane, all the way to here, which is the end of August, beginning of September, 1996, where we had a phone call with Nano Systems and some materials were exchanged and we had a discussion.

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In between that, it shows that within these four years, we were actually working hard on the development of Abraxane during that time period. And there were some critical milestones achieved during that time period. I will just point to a few here.

The first was in February, 1993, we filed our own patent, which is called the '686 patent, that covers cross-linking of albumin-based paclitaxel nanoparticles. Also, in February, 1995, we actually achieved a formulation of Abraxane which was amorphous. And we believed that it was amorphous and I have this notation in my lab notebook. We continued further development. We, in fact, contacted the FDA in April of 1996 to tell them that we had developed this novel form of paclitaxel and that we were wanting to go into the clinic with that. And we were able to scale up the process to make large amounts, and we put together a clinical plan with the FDA and we, in fact, confirmed that the particles were amorphous.

This was all occurring before we ever talked to Nano Systems.

Q. Dr. Desai, you have used that word "scale up" a couple times. Can you explain what that means?

A. Yes. That is a common word that's used in the pharmaceutical industry. When you first start with a new preparation of a drug, for example, and you start in the lab

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on the lab bench and you're working on very small test tube quantities of material. But, eventually, in thinking of going into the clinic, thinking of testing these, the drug in human patients and then eventually going commercial to sell the drug, you need to, of course, scale up, that is, you need to be able to not just manufacture in a test tube but maybe make it in large vats of material.

That is what scale up is all about.

Q. Let's take a look at some documents from the development of Abraxane.

What is JX-31, Dr. Desai?

A. This is an entry from my lab notebook. Pardon my handwriting. But I will try and explain to you what's there.

This is October of 1992 from my lab notebook. This says, the title says, Albumin MIC, which is short for microcapsules, with the drug Taxol, with Taxol.

Here, what we have done is -- this is -- we have created these nanoparticles here, which we call prunes. The reason we use the word "prunes," I will explain that to you in a minute, but the process here is using the solvent as you saw in that little animation. And, incidentally, we use the solvent benzene in this case. We made the little particles of the drug by pulling the solvent off, or evaporating it, very much like was seen in that animation.

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This resulted in solvent precipitation or precipitation of the amorphous particles from the solvent.

Then we took this material, which is the nanoparticles, and we actually put it into animals at this early stage in 1992. So this refers to two rats here that were tested with this particular formulation back in 1992.

Q. You mentioned prunes, Dr. Desai. What is a prune?

A. Well, that's sort of a nickname that we gave the structure that we were trying to create, because, at that point, we imagined it to be sort of spherical in nature, but the process that we were using was to first use the solvent and make the droplets. Then we were evaporating, or, in fact, drying out that process.

It is like, think of a grape that you dry out and you get a raisin or a plum that you dry out and you get a prune. So I guess we affectionately called this structure we were making a prune.

Q. You mentioned your '686 patent. Let's take a quick look at that.

This is DX-552. Can you just explain to the jury what the title of that patent is referring to, Delivery of Water-Insoluble Agents?

A. Yes. This patent covers, this is the first patent that we filed on Abraxane back in February 1993. And this patent, the title of it covers water-insoluble

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pharmacologically active agents, which means water-insoluble drugs. And useful compositions and methods for invivo delivery, meaning methods for giving this drug to an animal or humans. That's what we covered in this patent.

Q. You are an inventor on this patent?

A. Yes, I am an inventor.

Q. Let's take a look at Column 4, Lines 18 to 33.

Were you putting in your patent applications some of the work you were actually undertaking at the company you were then at, I guess it was called?

A. VivoRx.

Q. What is described, what does this reflect about capturing what you guys were thinking about at that time?

A. Well, this is in the section of the invention that talks about the detailed description. It gives a summary of what the invention is all about. Maybe if we can sort of expand this box a little bit.

So this invention talks about a polymeric shell, and the polymer here that we used is albumin, is the molecule we used, so we are creating a shell of albumin. Around a, particles of albumin, that is less than about ten microns, and the important thing that is covered in this patent is that this polymeric shell that is around the particles is cross-linked, and that the cross-linked -- that is, these molecules are linked together by a very specific

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linkage called the disulfide bond. So we cover that aspect of the invention in this patent.
Q. Let's take a look at JX-28.
What is JX-28, Dr. Desai?
A. This is an electron micrograph. So we did electron microscopy, looking at very high magnifications, to look at our particles. This was, again, done in the early days. So this gives an indication of what the particle looked like. It was more or less spherical, and it had a thin shell, which is not very visible, unfortunately, on this. But this shell, we could actually measure the thickness of that cross-linked shell. We have indicated that here, that the cross-linked shell was only about 25 nanometers thick. So it's a very thin layer. But it's there and it's cross-linked.
Q. Let's go back to the patent. What sizes were you thinking about in this time period?
A. In the time period when we filed the patent, we were thinking that the useful sizes of these particles could be in a range of about .1 micron to about five micron. Again, .1 micron is 100 nanometers, the same thing, all the way to five micron, which is 5,000 nanometers. That is the size range we were thinking of.
Q. Let's go back to the face page of the '686 patent.
Was there anything else interesting about the

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face page here, Dr. Desai?
A. Yes. If we can blow up that part right there. It was very interesting that the primary examiner and the assistant examiner -- these are examiners at the United States Patent Office in Washington, D.C., are Thurman Page and William Benston.
Q. Let's turn to JX-21 at 0525198.
What is interesting about this lab notebook entry, Dr. Desai?
A. This lab notebook entry is from early 1995. I believe the date on this was about February 1995. Here, we use the word again "prunes." But what we are testing here is a couple of things. And, in fact, this was a series of experiments. There are two experiments in this series. We were trying to evaluate one condition of making the particles. And that particular condition is the evaporation step.
When you do that evaporation step that removes the solvent, you can either remove it slowly over time or you can remove it in a flash, very quickly.
We were trying to see if that affected the outcome, that is, what kind of particles we would get if we did that slowly versus we did that very quickly.
So this first experiment talks about removing the solvent, in this case it's ethyl acetate, that is the

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short form for that, it is removed very slowly.
What I also note here, by removing it slowly, we give it time for crystallization. That is sort of a well-known process, that when you evaporate things slowly, the drug or the molecule that is inside has time to crystallize and make its crystal.
So we tested that here. And I think the results are shown on the next page.
Q. Let's go to the next page, please.
A. That is over here. So here is that first experiment, and then we do the evaporation very slowly. And this, what I have written here is the microscope, meaning I am looking through the microscope and observing what we see. We see these needle shapes, that I have drawn here, these are the characteristic needle shapes of paclitaxel crystals.
I confirm that by using polarizing microscopy, which is a type of microscopy where you use a polarizing lens, so you can tell if these things are crystals or not crystals.
Under the polarizer, I make the notation that these are mostly crystals. So what I proved here in this experiment is that if you evaporate slowly, if you take the solvent out slowly, you are giving time for crystallization and you will end up with crystals.
This is exactly what we were steering away from.

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Q. Let's go back to the previous page.
A. This was the second experiment in the series. So I have written here in my notebook, Redo the experiment and give no time for crystallization. So we wanted to do that evaporation very quickly to see if we can completely avoid formation of the crystals.
The results are, we have two pages down, on this page, this is blown out here, my observations, with the second experiment, and I looked through the microscope, and I see, I make a notation that most of the Taxol is probably amorphous. And that this is a good result. Meaning that this is what we were after right from the beginning. And we seem to have achieved this goal in this experiment.
Here we have now determined that at least one condition of the process, that is, evaporation, a very important step in the process, and if you do take time and do it slowly, you are going end up with crystals, which is not what we want. So you have to do it very quickly and prevent the crystal formulation.
Q. We are looking at Page 78 and 80 of your notebook, 198 and 200.
A. Yes, that's correct.
Q. Dr. Desai, let's go back to the timeline. Where are we now in the development of what became Abraxane as you were just describing to us?

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Q. Let's take a look at JX-7.
What is JX-7, Dr. Desai?
A. This is a notebook of one of the scientific staff that worked for me. His name is Shlomo Magdassi.
Q. Let's move to Page 462, please.
Dr. Magdassi was in what capacity as compared with you in the organization at this time?
A. He reported to me. Actually, he was a professor from Israel who was working with us at the time. And he is an expert in colloidal science. That is his field. He reported to me for that period of time.
Q. Let's take a look at 462.
What is his notebook reflecting here, Dr. Desai?
A. Again, there is a lot of information here. This is results of testing of several formulations that he was working on of our nanoparticles. He was also trying and testing all these different conditions to try and find the best condition.
And the focus in this testing is really looking at the size aspect. So after each preparation was made, you would measure the size by this instrument that's called laser light scattering. It is a technique that is used to measure size.
Clearly, at this time, you see several different numbers here. All of these numbers are the size in

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nanometers. There were several experiments here that show 130 nanometers, 150, 140. So what we are trying to show you is that, at that time, we had achieved, more or less, our target of being at about 150 nanometers or below.
Roughly around 150 nanometers, as I explained, is important for the filtration. So we have to get the particles through the filter in order to sterilize them.
So it needs to be well below the 200 nanometer range.
Q. What is the date of this work?
A. This is dated July 19th, 1996.
Q. You confirmed that?
A. Yes, through the previous page, I think, of this notebook.
Q. Let's take a look, again, at your notebook, JX-21, at 296.
What are you describing here, Dr. Desai?
A. Okay. This is, again, we sent samples out for testing, as I mentioned before. This is one of those institutes that we sent the testing samples to, it's called Southern Research Institute, it's a famous institute in Alabama, Birmingham, Alabama, that does these types of tests in animals. It clearly indicates that we have 150-nanometer preparation of Capxol that we are going to send to them for testing.

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Q. You dated this to July 22, 1996?
A. That's correct (did he say 22?)
Q. Let's take a look at 298, July 15th, 1996.
What are you showing here?
A. Here I am showing several notations I put down in my notebook. This indicates we are ready for scale up. That is, as I mentioned, going from the small laboratory scale to the larger scale that was more important for actual use in clinical trials. I make that notation there that we have to scale up from the small volume, 100 cc, 100 milliliters.
I am talking about a preparation of a package for the FDA. And that I am giving about a month for myself to prepare the package, that it should be ready in about a month, and that I need to call the FDA to set up what's known as a pre-IND meeting. That is, "IND" stands for investigational new drug.
When you first approach the FDA that you are ready to do testing in the clinical setting, that is in human patients, you need to compile a big package for the FDA with all the information.
So I wanted to set up that meeting. That's what this refers to.
Q. Let's go to Page 329 of JX-21. August 27, 1996.
What are you describing here?
A. This is some results of those scale up experiments

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that I referred to in the previous notebook page, and that we have actually conducted those experiments, and those scale up experiments result in a size of about 150 nanometers. And I noted that with an exclamation point here, indicating that we seem to have achieved our target when we scaled up from the small scale to the larger scale.
Q. Had you done that work that is reflected here before you first received information from Nano Systems?
A. Yes, definitely. This is August 27, 1996. This is before we ever received any information from Nano Systems.
Q. And the "this" is the work that led up to this notebook entry?
A. Correct. This is the scaleup work. It talks about the different conditions, how much Taxol is in the preparation. There are some details about what we did, actually did in the experiment.
Q. Let's take a look now, still in JX-21, still in your notebook, at Page 325.
What were you describing here, Dr. Desai?
A. In this notebook page, which is dated August 26 of 1996, after we had made the formulation of what we believed were amorphous nanoparticles, we wanted to confirm that by a technique that's known as x-ray powder diffraction. And this is a technique that is used all over the pharmaceutical industry, it's a standard technique that's used to determine

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whether a material is crystalline or whether it's amorphous.
So we utilized that very same technique, x-ray powder diffraction, and tested our own samples. And this was done by a third party. We sent out the samples for analysis.
This were the results. The results are actually dated 23rd August of 1996. So that's when the results became available.
I will just explain to you briefly what this means.
The number of curves here, there is four different samples. The first sample is just straight paclitaxel from the manufacturer. So we haven't done anything to that sample. We take the sample from the manufacturer. This is the thing that goes into our process, which ultimately becomes the nanoparticles.
So that sample shows all these peaks, which you can see, they are sharp peaks here. And that represents crystalline character. So it is absolutely clear that this material has crystalline material in it.
On the other hand, the final curve up here is the curve that represents the analysis on our nanoparticles, what we call Capxol preparation or what ultimately became Abraxane.
There is a complete lack of sharp peaks,

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indicating that it is amorphous. I have noted here that amorphous materials show broad humps as opposed to these sharp peaks.
So the conclusion of this experiment is that the paclitaxel in our preparation is amorphous and we were able to confirm it with a standard technique by August 26th of 1996.
Q. At some point, you came in contact with NanoSystems?
A. That's correct.
Q. How did that happen?
A. I saw a presentation by them at a scientific meeting. And then I thought it was very interesting technology. As, in my normal way, when I am interested about a scientific idea or presentation, I went and talked to the person who was presenting, and I said, Maybe we should have some discussions, there could be some collaborations, et cetera. That is basically the first contact with Nano Systems.
Q. Let's take a look at JX-28. What is this document, Dr. Desai? You have it in your notebook if you want to look at it?
A. This appears to be a fax transmittal on the 21st of August of 1996. And this is from Nano Systems to us. I believe this is the confidentiality agreement that we ended up signing.
Q. Let's take a look at JX-20 at 602. What is this,

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Dr. Desai?
A. This is the cover page of the Nano Systems packet of information that was sent to us, and that's why I indicated here "Nano Systems."
Q. This is from your files?
A. Yes. I found it in my files when I was doing discovery for this lawsuit.
Q. So let's take a look at 625 and 626 -- did we miss this? Let's go -- let's have that back up.
Was this a one-way or two-way confidentiality agreement, Dr. Desai?
A. As it states in this part of the agreement, each party may provide the other with certain information which the submitter considers confidential.
This was a two-way agreement. So Nano Systems was providing us with some information. We were providing them with some information.
So there was an exchange of information.
Q. So now let's go back to JX-20 and 625 and 626.
These are some slides from that presentation, Dr. Desai?
A. Yes. Correct. This is the information that was provided to us by Nano Systems. It was a packet of slides. There were several slides in the presentation. These are a few of them.

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Q. What is the date on the slide, sir?
A. The date on the slide is, it's August 28 of 1996.
Q. Did you study these slides and develop an understanding of what Elan was seeking to convey by them?
A. Yes, definitely. We looked at these slides carefully, and I formed an impression of what their technology was all about, as indicated in these materials.
While they are bringing that up, my impression, after looking at that technology, which I, of course, appreciated --
MR. SCHEVE: Your Honor, excuse me. There is no question pending.
BY MR. JACOBS:
Q. What was the impression that you formed, while we are bringing that up, Dr. Desai?
A. As indicated in this slide here, if you can blow that up, so they talk about parenteral NanoCrystal applications. Clearly, this technology was focused on crystals in the nano form, so small crystals. The key features were outlined in this slide, A, B, C, D.
The thing that I looked at and formed an impression on me was it's a platform of technology to restrict the compound to the vasculature. So their focus was to engineer their NanoCrystals, but when administered into an animal or human, the goal was to keep that

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guidelines for the FDA, we, in fact, hired him as a consultant to help us with that aspect.

And this "30K" refers to a discussion between me and Patrick -- in fact, this was Patrick's handwriting, where he wrote down, 30K, that we would pay him 30,000 for his consulting efforts for Abraxis.

Q. Patrick is Dr. Soon-Shiong?

A. Yes. Patrick is Dr. Patrick Soon-Shiong, who I worked for at the time.

Q. When did you hire Raj Selvaraj?

A. Raj Selvaraj we hired more than, I think it was three years -- this is in 1996, so this is three years later, in 1999, I think it was May 1999.

Q. Now, if you look at the primary examiner and the assistant examiner on the Nano Systems patent, what did you notice?

A. Well, it was very interesting because I noticed that the primary examiner -- these are the people in the United States Patent Office who look at patents and decide ultimately whether these patents can be issued as patents.

So Thurman Page and William Benston are the same two examiners who examined our patents.

And our patents were also filed in the early part of the '90s, 1993 time frame. They issued in 1995.

It was interesting to note this coincidence,

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that the same people in the Patent Office were looking at the Nano Systems patent and they were looking at our patent at the same time.

Q. Dr. Desai, you mentioned earlier an IND, an Investigational New Drug Application for Abraxane.

When did you file your IND?

A. The IND was filed in, we first had the pre-IND with the FDA. That is a standard process. That was in December '96. Then we worked on several things after that, where we had been asked to do some additional animal studies. And we ultimately filed the IND, which is the actual final application, in May of, I believe it was May of 1998.

Q. And then you, at some point, filed an NDA, a New Drug Application?

A. That's correct.

Q. When did you file that?

A. So the New Drug Application is the final application. After you have finished all your clinical testing and you are ready for commercialization, you file a huge packet of information. That's called a New Drug Application. So it comes several years after the IND application. That application was filed, NDA application, was in, I believe in 2004.

Q. Let's take a look, JX-64.

What is JX-64, Dr. Desai?

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A. This is the cover page of an application for the new drug Abraxane that we had filed with the FDA.

Q. Could you give the jury a sense of what one of these things looks like if you were to assemble it in a single place, one of these NDAs?

A. Well, it represents a huge amount of testing that is done, because to get a new drug out into the market, you need to do all of the clinical testing, you need to do all of the toxicity testing in animals, you need to show your manufacturing processes in every detail so the FDA can look at this and analyze it. It would probably fill the whole side of this courtroom if you were to put it all together.

Q. Let's take a look at 117473 of JX-6.

What is this, Dr. Desai?

A. This is one small part of that huge package of information. And this is a report that did several characterization studies on Abraxane. This is the Table of Contents. So it lists here things like x-ray diffraction, differential scanning calorimetry, electron microscopy, et cetera, all the different techniques that we used to analyze Abraxane to understand its physical form and its chemical features.

Q. Some of these -- actually, I should step back.

Did you participation in the preparation of this application?

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A. Yes, I did, actually.

Q. How heavily were you involved?

A. I was very heavily involved right from the beginning.

Q. Have you had personal experience with the techniques listed under physical analysis, or some of them?

A. Yes. I have had personal experience with some of them. I have, myself, done electron microscopy as part of my graduate career and myself done differential scanning calorimetry as part of my graduate career and have published on these techniques. And I, myself, have done not x-ray diffraction but a very similar technique that's called x-ray photo electron spectroscopy, which, in that sense, uses the same principles of using x-rays to analyze samples, and I published on those techniques as well.

Q. Let's go to 483 of 484 of your NDA.

What do these pages represent, Dr. Desai?

A. This is part of that characterization report that focuses on the x-ray diffraction testing, which, as I mentioned, is the standard method of testing or to analyze if the material is crystalline or not.

Q. What are you describing here? What are you representing by this information?

A. Well, it says here that there were different lots of material. We talk about stability lots and there are numbers here. Each one of these numbers represents a

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different batch of Abraxane that was prepared at a different time. And each of these are tested under rigorous conditions when you do stability. The goal is to test them at high temperatures, like 40 degrees, at very high humidity, like 75-percent humidity. The whole idea is to stretch these samples as much as you can, to see, under the worst conditions, what could happen.

So this ensures the stability of your drug when it is really out in the field, it may be transported here or there under very rigorous conditions, et cetera.

So this is part of the stability testing. As part of the stability testing, we looked at the crystalline or amorphous character. And we were -- so that was the x-ray diffraction testing that was done here.

Q. What was the result, what did you understand the results of these experiments to show?

A. Well, the results are highlighted in the conclusion. And, really, the first part of it says it all, that the nanoparticles of ABI-007, which is a code name we use for Abraxane, are amorphous. So under these rigorous conditions, which included testing over six months, where you store these samples at these high temperatures and high humidities and take vials and put them upside-down and do all kinds of things, that even after those rigorous conditions, after long periods of time, that the particles

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remain amorphous and that was the conclusion of this testing.

Q. Let's review 485 to 486, the next couple of pages.

What is this, what are these pages showing?

A. This is similar to the x-ray diffraction patterns that I have shown you just a little bit earlier which were in my notebook from 1996. This was testing done much later at the time of the NDA filing. This was included in the packet of information that was sent to the government, the FDA. Same type of test. This is the starting material. That is the raw paclitaxel drug which we get from the manufacturer, clearly shows you all these sharp peaks, indicating crystallinity.

On the other hand, these top two represent ABI-007, which is Abraxane that is tested under the different stability conditions.

And there is no sharp peak here. There are these broad humps, which are characteristic of amorphous material. So none of these materials are crystalline.

Q. Let's look at DX-41, Dr. Desai. Can you take a look at DX-41 in your binder, please.

Can you tell us what DX-41 represents, Dr. Desai?

A. Yes. DX-41 is a report of results on x-ray diffraction testing. And this was done not in house, in our

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company, but these samples were sent out to a laboratory called Micron, Inc. And they were tested for x-ray diffraction to show if they were amorphous or crystalline.

This is pages after pages of information on several different lots or batches of Abraxane that were produced. And every piece of data that's in there shows that each Abraxane lot that is tested was amorphous.

Q. How many times has Abraxis sent out samples for x-ray powder diffraction testing?

A. I don't know the number. But probably 20 or more, maybe more than 20 times. A lot of those samples are in this exhibit.

Q. What have the results shown in each case?

A. In each and every case, they have always shown amorphous results. There is no evidence of crystallinity in Abraxane.

Q. Let's just take a look at one page out of this, 103. So this is in May of 2006. What is this document?

A. This is the sign off sheet on the final report that resulted from all of this testing of Abraxane commercial and stability lots.

And there is a number of signatories here. I am one of them. And by signing here, you indicate that you agree with what's in the document and you agree with the conclusions and you have reviewed the testing. I had

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reviewed all the report. And I agreed with the testing and the conclusions that were in this report.

Q. 109 of DX-41, Mr. Broyles.

What did the report conclude?

A. So the report, the conclusion is very much similar to the one we saw earlier, that nanoparticles of Abraxane are amorphous, and that, when tested, these particular lots are tested, that it shows that Abraxane is amorphous.

Moreover, there was another aspect to this. We were manufacturing Abraxane at two different manufacturing factories, or sites. One was Ruby Street in Chicago. Another was Grand Island in New York.

We tested the product, Abraxane, from both of these sites, independently, and it showed that it didn't matter which site this came from, they were equivalent, and, in fact, no matter how you manufactured it, whether one site or the other, they are still amorphous after all the stability testing.

Q. Do you regard this as an achievement?

A. Actually, I do. And this is very interesting, because typically, amorphous materials are not very stable. And they can change over time, just because of their amorphous character. This showed under rigorous conditions, with that albumin present to help bolster the structure of these nanoparticles, that these nanoparticle character remain

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the sample, but it's actually a grid on which the samples are mounted.

What we are looking at only are these little spherical dots here. Some of them are spherical. Some of them are sort of irregular. If you remember, I explained to you how these particles are formed, that you start with the droplets, you have the solvent in there, and then you evaporate, so then you sort of shrink the droplets and you form these prunes.

So they are slightly irregular shaped but still, more or less, somewhat spherical. You can, in fact, see that here, not only some nice spherical, round shapes, but there is some prune-like shapes, if you will.

Q. The prune-like shapes, are they indicative to you of crystallinity?

A. No, not at all.

MR. SCHEVE: Your Honor, I object. This is now crossing the line to expert testimony.

MR. JACOBS: I am asking him for the conclusion he drew, Your Honor.

THE COURT: See counsel.

(The following took place at sidebar.)

THE COURT: Why isn't that objection well-taken, Mr. Jacobs?

MR. JACOBS: Because he drew a conclusion based

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on this work. And Abraxis has made representations based on this work about what you can glean from this. This is very much part of his analysis of his own particles.

THE COURT: You are saying as a matter of fact he drew a conclusion. He is not offering an opinion as an expert.

MR. SCHEVE: He testified in his deposition that the only test that they relied upon for crystallinity was x-ray powder diffraction.

Now he is asking for an opinion of whether these SEM and cryo-TEM images provide a basis for him to conclude it is crystalline. That is clearly an opinion.

MR. JACOBS: It is true he did rely, because even as their own expert testified, these are suggestive. But he will testify that he drew the conclusion that this was consistent with x-ray powder diffraction at the time he saw them.

THE COURT: Do you object to him testifying as a matter of fact that that's what happened, that's the conclusion he drew?

MR. SCHEVE: He asked him the question in present day terms, Your Honor, which is it's formed as an opinion. He testified in his deposition, as I will show, he concedes, I am not an expert in any of these techniques.

THE COURT: You will cross-examine him on that,

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if he rephrases there.

MR. SCHEVE: It needs to be rephrased.

(End of sidebar conference.)

THE COURT: If you could rephrase.

BY MR. JACOBS:

Q. On or about the time you saw these pictures, did you form a conclusion about what these pictures represented by way of crystallinity or amorphousness?

A. Well, this technique, the microscopy technique, I should say, is not a definitive technique for measuring crystals or the lack thereof. You really have to look at what these particles, what the shape of these particles and all of that; because x-ray diffraction is the definitive technique. But sometimes you can make some assumptions from what you see. And there may be suggestions.

From my background, all those years I worked on paclitaxel, any time I had seen paclitaxel crystals, they were always needle shaped. That is characteristic of paclitaxel. And that is published, also, by other people.

So when I looked at this, I see no needle shapes. My assumption is, Okay, this looks like what we think it is. These are the little round particles of Abraxane.

To me, even sitting here today, this does not look crystalline.

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MR. SCHEVE: Objection, Your Honor. Move that that be stricken.

THE COURT: I will strike that last part of the response. Ask an additional question, if you can.

MR. JACOBS: I don't need to, Your Honor. Thank you.

THE COURT: All right.

BY MR. JACOBS:

Q. During this lawsuit, Dr. Desai, Elan has pointed to snapshots of notebook pages from people working under you on Abraxane where there are references to crystals. I would like to look at a couple of those and ask you to tell us what was actually going on in those instances. Let's take a look at PX-19 at 292, a slide from Elan's opening presentation.

Can you tell us, based on your work with Nilesh Ron, what is going on in this notebook page?

A. Nilesh Ron was one of the members of my group. He reported to me and he did a lot of testing on Abraxane, like many of the other members of the team.

So here, he is testing -- can we blow this up, please?

I believe -- this is a lot number. This is a specific lot of Abraxane that was produced. This is, this lot was manufactured in 1997, on July 2nd. The reason I

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remember this lot very well is this was the first lot of Abraxane that we produced at the 20-liter scale.

We had done some scale up and increase in size. This is the first time we went to 20 liters.

Q. A liter is about how much, Dr. Desai?

A. A liter is about maybe that much. (Indicating.) Imagine 20 of those in a big vessel.

So this is the first time we had produced this lot. The second reason I remember this is because this was over the July 4th weekend. We spent the entire weekend sequestered in our labs making this first lot, this large lot of Abraxane.

When this was subsequently tested, after we made it, in fact, to our dismay, because we had put so much effort into it, it was found to have very high levels of the solvent that we were using in that process.

And we ended up rejecting that lot because we had certain criteria of what we mean by Abraxane, and to meet those criteria, it was way above, I don't remember the exact number, but probably 20, 30 times above the allowable limit for that solvent, which means it could be toxic if we put it into patients.

Based on that criteria, we rejected this lot. So this did not meet the criteria for Abraxane. And because we were not able to evaporate out all the solvent that we

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wanted, something clearly went wrong in the process. This was a rejected lot. We threw it away.

Subsequently, we also were interested in it. And, in fact, three years later, I believe, which is when this notebook is dated, in 2000, some of my colleagues were testing this. And they found a couple of things. They found the presence of some crystals. In fact, during the course of that testing that they did, it was also inadvertently, by mistake, contaminated. I think the samples were left on the lab bench.

So you see where it says "moldy." And, really, I think you can't really draw any conclusions out of this, other than they observed some crystals and other than there was a lot of solvent present, which, in Abraxane, it has to be at very low ppm levels.

Q. Does this have anything to do, in your judgment, Dr. Desai, with the question of whether Abraxane has crystals in it?

MR. SCHEVE: Objection, Your Honor. That is asking for opinion.

THE COURT: Rephrase the question, please.

BY MR. JACOBS:

Q. What happened to this sample, in the end?

A. Well, this sample was thrown away. As I mentioned, it was a rejected sample. It was never used. Just sort of

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stayed there in the back of our labs. At some point, we were testing some of it much later. But, clearly, this is not Abraxane as we know it today.

Q. Let's take a look at 397, 705 to 706. That is PX-397, 705 to 706.

Leslie Louie is the person doing the work on these notebook pages. She reports, for example, in 2000, few random crystals.

What is your understanding of what was going on in this work, Dr. Desai?

A. Okay. Can we expand those pages so I can look at them? Look at the individual pages.

Q. Let me ask you a general question, first of all. What kind of work goes on at Abraxis that is not the actual testing, it's different from the actual testing of Abraxane?

A. Well, we are a research group. I run the research group at Abraxis. These people whose notebooks you are seeing, they were part of the group. As part of ongoing research, we continuously are testing, even testing today, different drugs, different formulations, different aspects.

So even with Abraxane, once we got even to the clinical trials, we were constantly trying to further improve, to look at all the possible variations, to make sure we ultimately ended up with the right formulation.

So in these notebooks, this is one of those

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entries. She mentions, Few random crystals. What I would like to point out is, again, this is that same lot that we just talked about that had very high solvent levels. This is the lot we made over that July 4th weekend. And it had the same issues, that it had too much solvent present and we had rejected it and we had seen some crystals in that lot.

Q. Let's go to the next page. I could hand you the whole notebook if that would help you.

A. Sure.

Q. So what else do we see on these pages that Elan highlighted for the jury, Dr. Desai?

A. Okay. So, again, there is some evidence of crystals here. Again, this is testing on that same lot. So this is just further information. This was being tested over time, just to see what would happen if we just let the vial sit there over time, after you reconstitute the vial, that is put in saline, and re-disperse the particles and then let the vial sit, then, periodically, you would come back to look at the particles and see if there was any changes.

THE COURT: Doctor, this is Abraxis testing?

THE WITNESS: Yes.

THE COURT: I think you said Elan.

MR. JACOBS: That Elan showed to the jury.

THE COURT: I misunderstood. Sorry.

THE WITNESS: Yes, this is in our notebooks.

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Again, the appearance of a few crystals. This, again, is that same lot that we just talked about, which had very high solvent levels.
BY MR. JACOBS:
Q. This is the lot that you did what?
A. We rejected this lot.
Q. Let's go back to that first slide. There are a -- have you covered all of the entries on here, Dr. Desai?
A. I think we can see that. I will just look at it in my notebook.
Q. It's PX-400, is what is represented there. It's from 1996. Needle-like crystals. Leslie Louie?
A. Maybe we can just blow it up.
Okay. This talks about fluidizer recycling. Here we are using this equipment that is well-known to generate small particles. It is a fluid diver, or homogenizer is another word for it. Here we are testing the effect of different modifiers. We have albumin there. But in addition, we are adding other surface modifiers.
In this particular case, it's a surface modifier called Cremophor. When we add that in, we see the formulation of needle-like crystals.
So, again, this condition, or this particular experiment, showed us what not to do. That is, when we added Cremophor, we ended up with crystals. There is many

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experiments like this where we add other surface modifiers in, other than albumin, and we generate crystals. This is part of the full testing, to know what components we can add and not add to generate the formulation.
Q. And what you are describing, Dr. Desai, is that listed right on the page of the notebook?
A. It says right here, you can see needle-like crystals. And you can see we have added Cremophor, which is a surfactant or a known surface modifier.
Q. How does this bear -- how did this bear in 1996 on the development of Abraxane?
A. Well, in 1996, we were testing a lot of things. Amongst them, this was one aspect of it.
Since then, we completely moved away from using these type of agents, these type of surface modifiers. The only thing we use is just albumin with the drug. There is no other component.
Q. Let's turn to the topic of cross-linking, and let's take a look at JX-15. JX-15 is an e-mail that has been in court already, Dr. Desai. Let me quickly set it up. There is an e-mail from Zachary Yim to you dated January 17, 2006, and then an e-mail from you back to him just a few minutes later, and this is about cross-linking.
Can you explain what is going on in this exchange?

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A. Zachary Yim was a relatively new employee at that time. He worked for me in the formulation group, the group that does work on these type of products.
I had mentioned to him something about cross-linking. So he refers to that.
So I respond to him to clarify his thinking, to this e-mail, that when I was talking about cross-linking, I say, yes, but not heat-induced cross-linking, which is usually construed as denaturation. Denaturation is a word that is used in the context of proteins. And the most common example that all of you know is if you take egg white, which is albumin protein, and you heat it up, you make your scrambled eggs, you end up with denatured protein. So it turns into that fluff, white stuff.
So that's what I am talking about. When I am talking about cross-linking, I am not talking about heat-induced cross-linking, which is called denaturation or egg white, but I am talking about the mechanism that occurs on Abraxane.
I say that cross-linking results from unfolding of the protein, which is albumin, on the hydrophobic solvent/particle surface.
When I showed you that animation, as the protein is getting onto the surface of those droplets, that's what I was talking about. The protein accumulates on those

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droplets. And by its nature, it unfolds. And when it unfolds, this exposes certain chemical groups within the protein.
When that happens, the protein that's nearby, and also exposing its group, can link up, that's called a cross-linking. That's the process that I was describing.
And the cross-linking occurs through spontaneous disulfide interchange, that is, these disulfide bonds are the ones that link together.
Q. Do you believe that to be the mechanism by which cross-linking occurs on the surface modifier particles on Abraxane nanoparticles?
MR. SCHEVE: Objection, Your Honor. That calls for an expert opinion. The question was, "Do you believe"?
THE COURT: I didn't hear the question. Let's have a sidebar.
(The following took place at sidebar.)
(Question read as follows:
Q. Do you believe that to be the mechanism by which cross-linking occurs on the surface modifier particles on Abraxane nanoparticles?
MR. JACOBS: I am establishing that is a prior consistent statement. It is an e-mail he wrote in 2006. I could ask it a little differently. I could say, Do you believe that e-mail to be true.

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MR. SCHEVE: If it is being offered for prior consistent statement, clearly, there has to have been a confrontation with an inconsistent statement. There is no predicate.

THE COURT: There has been no attack. You can bring it back.

MR. JACOBS: Can I offer it as an explanation of his understanding of the mechanism by which he designed --

THE COURT: Now that you have told me you want to offer it as a prior consistent statement, I will say no. But you will get another chance, I am sure.

(End of sidebar.)

THE COURT: I am going sustain that objection.

BY MR. JACOBS:

Q. Let's turn to DX-112, please, Dr. Desai.

What is DX-112?

A. This is a summary table of evaluating of whether there is cross-linking on the Abraxane particle.

So there were several tests done, and all of the rows, all of the rows in this table represent different tests that were done to determine cross-linking. And they represent different lots of Abraxane, so Abraxane produced at different types.

Q. The jury has heard about what a dimer and oligomer and a polymer are, Dr. Desai.

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Could you jump to what you understood this, these test results to be demonstrating to you at Abraxis?

A. Yes. So we measured in this test the percentage of cross-linking, that is the percentage of where you have two or multiple or several albumin molecules linked together.

What we found in our testing, that there was at least 55 percent of the albumin that's on the surface of the particles that are cross-linked to each other in this technique.

Q. Did Abraxis do any experiments to investigate the nature of the cross-linking between albumin molecules on the surface of Abraxane?

A. Yes, we did.

Q. What this showed to you precisely was what, Dr. Desai, when you did it?

A. This helped us just quantify, give an idea of what percentage of all of albumin that is on the surface of the particle was cross-linked, and the determination that was made from this study was that 55 percent on average, through multiple, multiple batches, of that albumin on the surface is cross-linked.

Q. Now let's go to DX-76, and DX-76 is Voung Trieu's notebook. It has an initial date of July 2, 2007.

Let's go to 586.

In a nutshell, Dr. Desai, why did you do this

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experiment and what did you conclude from it?

A. Well, this was to look at the nature of the cross-linking. As I mentioned before, we believed that the cross-link was due to a special chemical bond called a disulfide bond. And we wanted to confirm that.

We had done earlier studies many years before to show that. But in the final Abraxane, as it is today, to confirm that, in fact, it was the disulfide link, we did this experiment.

And I will try and describe very briefly.

These bands here that you see represent monomer albumin, that is single molecules of albumin.

On the other hand, these large smears represent polymers, that is, large molecules of albumin that are linked together.

This experiment showed us, clearly, that these large polymers of albumin or cross-linked albumin existed in Abraxane, which sort of confirmed the previous experiments that we had done where we showed 55 percent cross-linking. And to confirm, in fact, it was disulfide links, we put in a special chemical that selectively breaks disulfide links.

So if you had those polymers all together, which is represented here, and you put the chemical in, you would now break these apart and reduce them. That's the reference to reduce, reduce them to the single band of monomeric

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albumin.

So this proves, in effect, two things; one which we had already proved before, that there are cross-linked albumins on the particle, and, two, that these cross-linked albumins are cross-linked through a disulfide bond.

Q. DX-70 at 17002, please.

Dr. Desai, your testimony before the FDA has come up in the trial so far and comments you made about Abraxane. So we need to look at that quickly.

October 10, 2006, did you give a presentation?

A. Yes, this was one of the presentations I made at the FDA nanotechnology meeting.

Q. In what capacity were you invited, or for what purpose were you invited?

A. Well, this was an open forum to discuss issues with nanotechnology drugs, because this was such a new field, emerging field.

We had put in, Abraxis, that is, had put in a request to be able to present that at that meeting. We were granted that request. That is the context of this meeting.

Q. Let's go to 202 and 203 of the transcript, 203 and 204 of the Bates pages, and you are quoted there as saying, We use the native albumin. And if you start cross-linking the albumin with chemicals and things, I think you might run into problems but we don't do that.

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injectable form, which is a form that can be administered into the vein of a patient, which is different from administering, say, a pill, which you take by mouth, because the standards are very different. There is a much higher standard of safety for a drug that you put into a vein as opposed to something you put into the body by mouth.

So that's what I was referring to.

Q. To summarize, Dr. Desai, this testimony that you gave today, in Abraxis' view, is the paclitaxel in Abraxane amorphous or crystalline?

A. In my view, it is amorphous.

Q. And do you believe that is important?

A. I believe that's very important, because it actually defines our technology. It allows the drug to get rapidly out of the bloodstream into the tumor. And we have demonstrated and proven the benefits of this in patients.

For example, in our clinical trials versus Taxol for breast cancer, twice the number, roughly twice the number of women with breast cancer responded to our drug as opposed to those who responded to Taxol.

We also showed a benefit in survival in these patients. And I think, clearly, and, luckily, we adopted that amorphous strategy, which allows the drug to get to the tumor where it really needs to act.

Q. And, in your view, is the human serum albumin on the

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surface of the Abraxane nanoparticles cross-linked or non-cross-linked?

MR. SCHEVE: Objection, Your Honor. That is clearly asking for an opinion.

THE COURT: I am going to sustain the objection as to the question as phrased.

BY MR. JACOBS:

Q. Did you intend to develop Abraxane so that it had surface modifier molecules that were cross-linked or non-cross-linked?

A. Clearly, our intention was to develop a cross-linked albumin on the surface of the particle. We wanted to use albumin because we felt it was a very important part of getting the drug out of the blood very quickly. In all the testing that we did, that I was personally involved with, we saw the presence of crosslinks.

Q. Did you believe that was important to your strategy for Abraxane?

A. Yes, absolutely. It's a key feature, we feel, on the aspect of keeping the particles stable. We demonstrated the particle is stable through rigorous testing. And the only reason that, I believe, that it is is because it has that cross-linked albumin and it also has a lot of the additional albumin, the tenfold excess of albumin, to keep it stable.

Q. Did the Nano Systems or Elan NanoCrystal work

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contribute anything to your development of Abraxane?

A. No. It did not contribute anything. In fact, it was clear that the pathways were completely different. I give them credit for developing the oral drugs that they did with their technology in the NanoCrystal form. Clearly, ours was a different approach.

Q. Did the Elan '363 patent, when you read it over in 1996, or whenever, contribute anything to the development of Abraxane?

A. No, it did not. It just showed that the approaches were quite different.

Q. In view of the understanding that you had developed over time about the path that Elan was on as compared with the path that Abraxis was on, what was your reaction when you were sued for patent infringement?

MR. SCHEVE: Objection, Your Honor.

THE COURT: Sustained. I think I know what you want to do, but you can't. Rephrase.

BY MR. JACOBS:

Q. When you were sued for patent infringement, what did you reflect back on in terms of your understanding of the Elan technology and what you had come to know about it?

A. Well, first of all, I was shocked to hear about a lawsuit, given that I had seen Elan's presentations at public meetings, given that I was familiar, to an extent,

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with their technology from the materials that were sent to us back in 1996, and given that we had always pursued independently a completely different path. Our path was amorphous and cross-linked. Their path was crystalline and non-cross-linked, as was clear from the reading of those patents and their presentations.

Really, it didn't make sense to me. I said, Well, where is this infringement? I didn't have any idea.

The second part of it was, I took it at a more personal level --

MR. SCHEVE: Objection, Your Honor.

THE COURT: No. I will permit him to continue. I recall similar testimony from Elan.

Go ahead.

THE WITNESS: So the second aspect that I was thinking was really more a personal level. I knew Gary Liversidge and I had taken special efforts when I organized certain symposia and chaired meetings in 1998 and in 2002 to invite Gary to speak at these symposia. These symposia were focused on drug delivery in general, where I had multiple speakers come together and present their scientific data.

I presented myself on our technology. Gary presented on his technology.

And, to me, this was almost like a slap in the face. After all these years where I have invited him, I

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recognized him as a good scientist coming to make presentations at meetings at the same venues that I was making presentations, it seemed impossible to me that he wouldn't be able to grasp the issue of the differences. Amorphous, crystalline, cross-linked, non-cross-linked.

So I really don't know what was behind this. But I think -- I was quite upset at the time.

MR. JACOBS: Thank you, Dr. Desai.

THE COURT: Mr. Scheve, you may cross-examine.

MR. SCHEVE: Would there be any opportunity for a short break?

THE COURT: Sure. Ladies and gentlemen, let's take a very short break.

(Jury leaves courtroom at 11:55 a.m.)

(Recess taken.)

MR. JACOBS: Before Mr. Scheve's cross, he represented when he played Dr. Desai's deposition testimony, that he would not be recrossing on topics that he played. And I just wanted to remind him.

MR. SCHEVE: I am not going to re-ask any questions I asked. There are clearly some things that I didn't ask that I hope to get into in cross.

THE COURT: All right. We will see.

(Jury enters courtroom at 12:05.)

THE COURT: Please take your seats.

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Mr. Scheve, you may cross-examine.

MR. SCHEVE: Thank you.

CROSS-EXAMINATION

BY MR. SCHEVE:

Q. Slide 67.

Excuse me.

MR. SCHEVE: Your Honor, I apologize. My thumbs again.

(Pause.)

MR. SCHEVE: It is not my thumbs. It's my eyes, Judge.

BY MR. SCHEVE:

Q. Doctor, I blocked you off, I apologize. Let me move this a little bit.

Thank you for being patient with me.

In examination by Mr. Jacobs, you made reference to some patents covering Abraxane. Is that correct?

A. Yes.

Q. Are you familiar with something called the Orange Book?

A. Yes, I am.

Q. Is the Orange Book a book in which you have to sign certain forms representing certain things to the FDA in order to get your patent listed there?

A. To my best understanding, the Orange Book is when you have a drug approved, you have to identify to the FDA which patents cover the drug. So you send them a list of the patents and there are some forms that are signed for that purpose.

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Q. This patent, the '868 patent, is that one of your more recent patents that's listed in the Orange Book as covering Abraxane?

A. Yes. I believe that's an Orange Book patent, that's correct.

Q. Isn't it true, sir, in your own patent covering Abraxane, it is stated in Column 8, quote, "While it's recognized that particles produced according to the invention can be either crystalline, amorphous, or a mixture thereof, it is generally preferred that the drug be present in the formulation in an amorphous form"?

A. Yes, I see that written in the patent.

Q. Is it also stated in your patent covering Abraxane, quote -- this is Column 32 -- "It is also known that as the loading of the drug and the formulation is increased, the tendency for crystallization also increases"?

A. I am not sure about that. If I can see that page, please.

Q. Okay. This would be Plaintiff's Exhibit 211, sir.

Do you have that notebook in front of you?

A. Which exhibit?

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Q. Plaintiff's Exhibit 211.

Did you find it, sir?

A. Yes.

Q. Could you go over to Column 32. Are you there, sir?

A. Yes.

Q. Do you see on the fifth line, quote, "It is also known that as the loading of the drug in a formulation is increased, the tendency towards crystallization also increases"?

A. Yes, I see that. That is in reference to as you try and pack more and more drug into the particles, at some point, you reach a limit, and then you may see crystallization beyond the limit.

Q. Let's go to the next patent you have, the '579.

Is it listed in the Orange Book as covering Abraxane?

A. I am not sure about the -- I don't remember all the numbers. Maybe you could just identify if it is or not.

Q. Are you familiar with this patent?

A. Can I see the face page, please?

Q. Certainly. It's in Defendant's Exhibit, this would be Abraxis' Exhibit 030. I am probably going to have to review back to one of those heavy notebooks. I apologize for the heavy lifting.

A. Did you say 030?

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Q. Preclinical means animal?
A. That's correct.
Q. Isn't it true that the first human study, the first batch wasn't made until March of 1998?
A. The first clinical batch, which was used in our clinical trials, as identified in this table, is down here. (Indicating.)
Q. Sir, isn't it true that you began using this very specific model of microfluidizer, the M 140 K, clear in the first week of February of 1997, 13 months before the first clinical batch?
A. Well, this data just represents the batches that were used for testing that was sent to the FDA, meaning if we did any animal tests, we had to identify which particular lots were used for the animal test.
So there was a lot of other development work that was going on at the same time, just in finding the right experimental conditions and to produce the product. So that information is not represented on this table. So I would say that even prior to this date, that we were using equipment such as this to produce nanoparticles of paclitaxel.
Q. Can you find it on this page, sir?
A. No, it wouldn't be on this page, because this reference the specific lots, as indicated in the title, that

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are used for preclinical, that is for animal studies, and for clinical studies.
Q. When was the first clinical study started, sir?
A. It was started in May of 1998.
Q. May of 1998?
A. I beg your pardon. The IND was filed in May '98. The clinical trials started in July.
Q. In July of '98?
A. Yes.
Q. This chart shows that the first Phase 1 batch was made in March of '98. Correct?
A. That's correct.
Q. The jury has seen a number of images of crystals.
Would you please put up No. 45.
Do you see this here, sir? Microscope! Needles! Pretty damn small! Normal sample-microscope-needles.
Do you see that, sir?
A. Can you tell me which exhibit it is?
Q. Yes, it's Joint Exhibit 62.
THE COURT: What's the Bates page, Mr. Scheve?
MR. SCHEVE: It ends in 191, Your Honor, I am sorry.
MR. JACOBS: Your Honor, we are having trouble tracking the exhibit and the Bates page.

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THE COURT: I am, too. JX-62?
MR. SCHEVE: 062.
THE COURT: I have that.
THE WITNESS: I don't think it's in here. All the Bates pages start with 527.
THE COURT: Yes. The last three numbers, Mr. Scheve, on the first page, 474 through -- well, it goes on from there.
MR. SCHEVE: Let me go forward. I apologize to the Court and the witness for that. I have it marked as that. If I am wrong, I stand corrected. I apologize.
BY MR. SCHEVE:
Q. Let's go forward to the next one, please.
Do you recall, sir, the discussion that you had with counsel for Abraxis about these references to crystals? I am now looking at Joint Exhibit 021.
A. Yes. This, I testified this morning about this particular example from my notebook.
Q. If we go forward in 1995, you will acknowledge, sir, that there are references to crystals being observed at different stages, in other words, some in the manufacturing process and then later on, actually, after they were reconstituted with saline solution.
Do you agree with that, sir?
A. No, actually, that is a mischaracterization.

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Let me clarify.
This is a notebook from Dr. Shlomo Magdassi's notebook, who used to work for me. In this particular case, on this date, this was in early '96, he is doing an experiment where he uses a surfactant, a surface active agent or a surface modifier. If somebody could blow this up. It says, Cremophor. I can see right here. He uses Cremophor in this formulation. Here we are, when I talked to you earlier about testing all kinds of different conditions, all kinds of different components, in this case, we are testing Cremophor for. The results that he got was crystals. And he identified right here that, "The problem: Crystal growth. How to prevent it"?
So this is clearly a result that we did not want or striving to get away from crystals. This is a condition that we did not want, using this particular component, Cremophor. And we completely steered away from that. In fact, in the patent that we filed later, in this very same year, which had the amorphous x-ray diffraction data in there, there are specific examples in that patent, this is one of the examples in there, it clearly shows and identifies that this is a technique that is not to be used for the invention. That the addition of surfactants should not be done.
Q. If we move forward, you told us here now that you

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The point is that if it's -- if an x-ray is penetrating a particle, scale wise, the x-ray may look like this, but that smallest particle, nanoparticle, looks like that. (Indicating.) So there is no chance that the x-ray is not going through the particle.

When you have a bigger particle that, in the context of the nanoparticle is even as big as this room, that x-ray is still going to go through and penetrate, no matter what.

Whether you have a big particle or a small particle, the x-ray is so much smaller than either of those that it makes no real difference to whether the particle is big or small.

Q. And the important point, then, in setting up the physical control was to match what to what in terms of the paclitaxel?

A. So the control was matched to Abraxane. So we had the same amount of paclitaxel in the control as we had in Abraxane.

For the control, we could see the crystalline peaks. And for Abraxane, we could not.

Q. Did you ever file a patent application with the x-ray powder diffraction data we just saw?

A. Yes. Soon after this data was obtained, we were already in the process of compiling a patent application,

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which, in fact, contained all of the information from the previous year or year and a half.

And within, I think, a month after we got this final definitive data, we filed the application.

This was literally the last piece of the information that went into the patent application.

Q. So let's pull up DX-605. Is this the patent application that led to the 5,916,596 patent that you were referring to, sir?

A. Yes. This is the exact one that we filed soon after we got the x-ray diffraction data.

Q. If you go to Example 13, does Example 13 of this patent reflect the x-ray pattern diffraction data that you just showed the jury?

A. Yes. It's that very same data that we obtained by August 23rd of that year.

Q. Did you report to the Patent Office your conclusion about amorphousness?

A. Yes. We, in fact, up here in the introduction to that data, we said that it is advantageous that the formulation contain the drug in amorphous form, because of the issue of bioavailability, which we have talked about a lot, that it dissolves very quickly, like cotton candy.

In this paragraph, we actually presented the data, Sample 1, Sample 2, Sample 3, Sample 4. Those are the

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four curves that you saw in that graph.

The conclusion here is that the presence of, in Sample 4, which is the formulated paclitaxel, showed no evidence of crystallinity which is characteristic of the standard raw material paclitaxel. And that was essentially the conclusion.

MR. JACOBS: Thank you. No further questions, Your Honor.

THE COURT: Thank you, Dr. Desai. You are excused.

THE WITNESS: Thank you.

(Witness excused.)

THE COURT: Ms. Kruze, who is your next witness.

MS. KRUZE: Dr. Mansoor Amiji.

...MANSOOR AMIJI, having been duly sworn as a witness, was examined and testified as follows...

DIRECT EXAMINATION

BY MS. KRUZE:

Q. Good afternoon, Dr. Amiji.

A. Good afternoon, Ms. Kruze.

Q. Could you please introduce yourself to the jury?

A. My name is Mansoor Amiji. I am a professor of pharmaceutical sciences at Northeastern University in Boston.

Q. Are you testifying here today as an expert witness?

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A. Yes, I am.

Q. What is your area of expertise?

A. I am in the pharmaceutical sciences department. I am an expert in the area of drug delivery systems, specifically, looking at nanoparticulate drug delivery, as well as nanotechnology applications in medical problems.

Also, my research interest is in polymeric biomaterials.

Q. Could you please turn to Defendant's Exhibit 474 in your binder. Mr. Broyles, if you could bring that up on the screen as well.

Would you identify this document for the jury?

A. Yes. This is a copy of my curriculum vitae, referred to as CV.

Q. And where did you do your undergraduate work?

A. I did my undergraduate work at Northeastern University, the same institution that I am currently employed at.

Q. And could you tell the jury if you have any advanced degrees?

A. Yes. I do. I have a doctoral philosophy degree, a Ph.D. degree in pharmaceutics from Purdue University.

Q. What was the subject of your dissertation?

A. I worked on an area called surface modification of biomaterials, specifically in the area of hydrophobic or

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in the area of nanomedicine.
We, at Northeastern, have received this grant from the National Cancer Institute and it is administered by the National Science Foundation. We have close to six or seven doctoral students a year, which is in their third year of this grant, so we have funded close 15 or 16 Ph.D. students.
Q. Let's turn to cross-linking. If we can bring up JX-81.
Dr. Amiji, do the claims of the '363 patent require that the surface modifier molecules in the nanoparticles be non-cross-linked?
A. Yes, it did. Here we can see clearly in the first claim of the '363, it says that the non-cross-linked surface modifier --
MR. SCHEVE: Your Honor, this term has been construed by Your Honor specifically. If he is saying that this is the construction, it clearly is not the Court's construction of the term.
THE COURT: Let me see counsel.
(The following took place at sidebar.)
THE COURT: What is your response?
MS. KRUZE: My very next slide is going to the patent talking about that. It is exactly what Your Honor says in the Court's claim construction.

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THE COURT: For the jury's benefit, we should avoid questions that might confuse them as to this issue. As we know, this has been hotly contested. The other side disagrees with my construction. They are entitled to. We don't want this fact-finder confused.
MS. KRUZE: No problem. I will just refer to how the patent talks about it. Or have him read from the patent.
THE COURT: I have construed contested terms.
MR. SCHEVE: Free of intermolecular cross-linkages.
MS. KRUZE: The very next slide is exactly that.
THE COURT: That is fine. I think the objection is to this slide.
MS. KRUZE: We will move directly on then.
THE COURT: So do you want to withdraw that question? Do you want to rephrase that question?
MS. KRUZE: Let me rephrase.
THE COURT: Do you want to move into the next slide?
MS. KRUZE: Yes.
(End of sidebar conference.)
BY MS. KRUZE:
Q. If you could bring up DD-33, which is based on JX-81.
Could you please read the highlighted term?

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A. Yes. So here, again, in the patent itself, it says, essentially, free of intermolecular cross-linkages.
Q. Let's move to DD-70. When protein molecules exist in a non-cross-linked form, what do scientists call them?
A. Here we have heard this already, the terms in this trial, when you have single protein molecules, these are called monomers. When protein molecules associate and form two protein molecules associate, that is called a dimer. When you have three, that is a trimer. Many would be an oligomer. Even more would be polymers.
Q. Are dimers another word for cross-links?
A. Yes. Anything, once the proteins associate with each other, dimers, trimers, oligomers, and polymers would be considered cross-linked.
Q. Are there different types of cross-linking?
A. Yes. There are two types of cross-linking. There is a physical cross-linking and what's called chemical or covalent cross-linking.
Q. What is an example of physical cross-linking?
A. The best example I can give is the favorite of our children, the gelatin. We make gelatin, we take gelatin, put it in warm water. You take that, put it in a mold, put it in the refrigerator and it gels and forms this squiggly gel. That is a physical cross-link because you have changed the temperature and now the gel has solidified.

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If you reverse that by heating that gel, it will become liquid again.
Q. You also mentioned chemical cross-linking.
What does that involve?
A. Chemical cross-linking involves formation of these bonds, what I would call covalent bonds.
Q. Does the '363 patent distinguish between those two different types of cross-linking?
A. No, it doesn't. The '363 patent was very clear in the fact that all types of cross-linking were included.
Q. Can you pull up DD-71, Mr. Broyles. This is a 1006 exhibit based on JX-81, JX-39 and DX-16.
What are these, Dr. Amiji?
A. Here are some of the comments from the patent file history, as well as the patent itself. Both the '363 patent and the '684 patent, and we have heard the '684 being referred to as the mother patent for the '363.
You look at different comments. Let's start with the first one on the top, where we see -- this pointer is probably blinking. I can't see from here.
The top one there is the '288 prior art. That's the Oppenheim prior art. It says, The particle comprised a cross-linked matrix of macro-molecules having the active material supported on or incorporated into the matrix.
Here, the '363 applicants were distinguishing

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their invention from this prior art, the Oppenheim prior art, based on the fact that it was cross-linked and theirs was not cross-linked.

The second comment, looking at the '294 patent, that's the Motoyama patent, again, the applicant distinguished their invention, the '363 applicants distinguished their invention based on the fact that the Motoyama patent had this crystallized carbohydrate matrix and these carbohydrates were cross-linked. Here is a case where it was physically cross-linked.

MS. KRUZE: Permission to approach the witness, Your Honor?

THE COURT: Yes.

BY MS. KRUZE:

Q. Dr. Amiji, is the albumin coating on the surface of Abraxane particles non-cross-linked?

A. No. The albumin coating, as we just heard, is significantly cross-linked on the surface of the Abraxane nanoparticle.

Q. How do you know?

A. I have looked at a number of different documents from Abraxis, documents that I have reviewed, experiments that I have reviewed. And all the documents that I have seen show that the surface of the Abraxane nanoparticle, the albumin that is on the surface, is substantially cross-linked.

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Q. Let's discuss some of those. Dr. Amiji, could you turn to DX-107 in your binder.

Mr. Broyles, could you bring that up on the screen?

What are these documents?

A. This first one is a document from Abraxis. It specifically deals with a test that is called size exclusion chromatography. It is a fancy way of saying sieving experiment. The only difference between this type of experiment and a standard sieve is a sieve separates from a particle and basically gets out smaller particles. In this case, the larger particles get out first.

What you see at the bottom in the table is the composition of the albumin that is present. Here we see the polymer, having approximately 38.9 percent of the composition, the oligomer having 3.8 percent of the composition, and the dimer having 11.3 percent of the composition.

If you add the three numbers, the total composition of the cross-linked albumin is going to be about 55 percent.

Q. Could you go back to the original screen.

When were these tests conducted?

A. These tests were conducted, it's in the upper part of the document, in January of 2003.

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Q. Before this lawsuit?

A. Yes, they were conducted before this lawsuit.

Q. Let's bring up DD-104, which is based on DX-112.

What is this?

A. So here, again, we see the -- there are, in this table that you see in this slide, the monomer, dimer, oligomer, and polymer compositions from 17 different experiments that were done with different lots of Abraxane on different days, in certain cases, with triplicate or duplicate experiments.

All of them report the fact that there is a high degree of cross-linking in the albumin with an average at the bottom there showing about 55 percent of the surface-bound albumin on the nanoparticle being cross-linked.

Q. Did you investigate the nature of these bonds, Dr. Amiji?

A. Yes, I did.

Q. Let's turn to DX-76. Could you tell the jury what this is?

A. This is the lab notebook of an Abraxis investigator by the name of Voung Trieu. It's dated July 2007.

Q. If you could turn to Page 10 of the document, also Bates numbered 523594. Could you explain to the jury what sorts of tests are on this page?

A. This is an experiment that was done at Abraxis to

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confirm the type of bond that's formed in these cross-linked albumins. What you see here is what we referred to in the protein chemistry art as polyacrylamide gel electrophoresis, abbreviation PAGE, P-A-G-E.

Basically, these gels are made from a polymer called polyacrylamide and proteins are then run in these gels to create various lanes.

So what you see are, in each of those, are the lanes of proteins, so 15, 17 and so forth. And the lane will have -- in this case, the lane is designated by this dye called coomassie blue or coomassie staining. That basically stains for the protein. It doesn't sustain the gel.

When these proteins are run, what you see in the non-reduced state, which is when the cross-linking still is in tact, is the fact that the protein has higher molecular weight. It's greater than the albumin control that you see down here.

All this on the top, the bands that you see on the top correspond to the higher molecular weight. Those are the cross-linked albumin.

When these same proteins are taken and run under reduced conditions, in this case, now, the disulfide bonds that form with these cross-links are intentionally broken with a chemical that breaks those bonds, and now we see that

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the lane that these proteins migrate to is exactly the same as albumin.

So you are intentionally breaking the bond, using this disulfide bond breaking agent, and the reduced condition, to get the free albumin back.

So based on these results, it's clear that the bonds that are formed in these cross-linked albumin is by disulfide bonds.

Q. Is this a typical type of experiment to determine the presence of disulfide or covalent bonds?

A. Yes, it is. It is actually the standard test that's used to confirm disulfide bonding.

Q. Did you review the lab notebooks and other materials that were associated with these experiments?

A. Yes. So, again, as I looked into the lab notebook, at one part of the lab notebook, there was a standard operating procedure that was used, and I reviewed that standard operating procedure, and it's very characteristic of the experiments that I have done for these types of analyses.

Q. Were these experiments conducted well?

A. They are conducted extremely well. I was able to find all the information that I needed to make my interpretation and my analysis and my conclusions.

Q. Let's bring up DD-75, Mr. Broyles. This is from Defendant's Exhibit 107.

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Have you reviewed any tests conducted by Elan or its experts regarding cross-linking?

A. No. I have not seen a single test done by Elan or its expert in regard to the cross-linking Abraxane.

Q. To the best of your knowledge, did any Elan expert conduct testing of cross-linking in Abraxane?

A. No. I have not seen a single test from an Elan expert on cross-linking in Abraxane.

Q. Were you here when Dr. Manning testified?

A. Yes, I was.

Q. Could you remind the jury who Dr. Manning was?

A. Dr. Manning was an expert from Elan's side. He testified on this area of cross-linking. I believe he was on the faculty at the University of Colorado. Currently, he has his own company. I believe it is called Legacy BioDesigns.

Q. Do you recall when Dr. Manning gave testimony regarding 15 to 20 percent cross-linking?

A. Yes, I do.

Q. What is the discrepancy between the number you were just talking about, 55 percent cross-linking, and the number he testified to, 20 percent?

A. So he was talking about the entire Abraxane formulation. As we just heard a few minutes back from Dr. Desai, Abraxane has this free albumin and there is also

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albumin that is on the surface of the nanoparticle.

When you look at the entire composition of Abraxane, there is about 20 percent cross-linking. However, when you look at the surface of the nanoparticle itself, it's actually got about five percent cross-linking.

Q. And which one is the relevant number for this analysis?

A. So the relevant number for this analysis is what's on the surface, because that's really what's in the claim of the '363 patent, how much of the surface modifier is on the surface of the nanoparticle.

Q. Let's go to JX-81, Claim 1. What is the language here that shows you the relevant amount?

A. If we look at the fifth line -- starting from the fourth line, it says, Non-Cross-Linked surface modifier adsorbed on the surface thereof.

What this refers to, clearly, in the claim language, that this is the surface of the nanoparticle.

Q. And have you reviewed any documents where Dr. Manning agrees with your analysis that the free albumin can be different than the absorbed albumin, for example?

A. Yes, I have. Actually, in review of various documents, I came across a chapter that Dr. Manning wrote that when the nanoparticle, or when you have surfaces, there is higher concentration of the protein on the surfaces, and

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Dr. Manning clearly identified that on surface of various hydrophobic surfaces, there is greater concentration, and, therefore, you do have this cross-linking phenomenon.

Q. Mr. Broyles, can we bring up DD-115 on the screen, and the source of this is PX-493.

Is this the article that you were referring to?

A. Yes. This is Manning article from 1992. It is more than a thousand times more concentrated protein in the surface than on the bulk phase. Bulk refers to basically the free albumin.

Because the albumin concentrates significantly on the surface, there is a higher probability of formation of this cross-linking, and that is really what we observed with Abraxane.

Q. Did Dr. Manning contest your opinion that 55 percent albumin is cross-linked in the nanoparticles?

A. No. I haven't seen a single comment or any opinions from Dr. Manning that contests that.

Q. Did Dr. Manning review any Elan tests when he was giving his testimony?

A. No, I don't believe Elan did any tests.

Q. Did you also hear his testimony that Abraxane behaves like a non-cross-linked particle?

A. Yes, I did.

Q. Did you agree with his opinion?

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A. Again, as we just heard a few minutes back, when you put Abraxane in the body, it dissociates very fast. Once it dissociates, the fact that these nanoparticles, and the disulfide bonds is not because you are putting a chemical in, it's formed because of the fact that there is inherent formation of these linkages in proteins, and that dissociates in the presence of blood. There is no discrepancy between the fact that albumin and Abraxane is able to go into blood very quickly and this idea that the surface is cross-linked.
Q. Bring up DD-75 again. In summary, Dr. Amiji, what is your opinion regarding cross-linking of the albumin on the surface of the Abraxane particles?
MR. SCHEVE: Objection to the form of the question, Your Honor.
THE COURT: Repeat the question.
BY MS. KRUZE:
Q. In summary, what is your opinion regarding cross-linking on the albumin on the surface of the Abraxane nanoparticles?
THE COURT: Form, you object to why?
MR. SCHEVE: There is a certain standard that has to be announced by the witness before the opinion becomes evidence or admissible as opposed to submittable. There has not been an appropriate question asked.

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THE COURT: Let's go to sidebar.
(The following took place at sidebar.)
THE COURT: I think Mr. Scheve is going with this you need to ask the witness, Do you have an opinion to a reasonable degree of scientific certainty.
MR. SCHEVE: That's part of what is missing.
THE COURT: Where else are you going, Mr. Scheve?
MR. SCHEVE: There is a series of two or three questions there that she has to ask and she hasn't done it. I am objecting for the record.
MS. KRUZE: I am sorry. This is my first time with an expert.
(End of sidebar conference.)
BY MS. KRUZE:
Q. Do you have an opinion, to a reasonable degree of scientific certainty, regarding non-cross-linking?
A. Yes, I do.
Q. What is your opinion regarding cross-linking of the albumin on the Abraxane nanoparticles?
A. I believe based on all the studies that I have analyzed and looked at and the conclusion that I draw that the surface of the Abraxane nanoparticle has substantial cross-linking of albumin.
Q. Let's turn to -- actually, one more question. Do you

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have an opinion regarding the nature of those cross-links to a reasonable degree of scientific certainty?
A. Yes, I do. I believe that the nature of the cross-linking in the Abraxane nanoparticle surface is through that disulfide bond.
Q. Let's turn to crystallinity. Could you turn to DDX-15. Could you tell the jury what this is?
A. Again here, we are looking at different comments from the patent abstract, the patent itself, the '363 patent.
Here, the patent, itself, distinguishes, that it is for crystalline anticancer agent. And crystalline phase differs from the amorphous phase.
Q. This comes from JX-81, for the record.
Dr. Amiji, is an amorphous drug equivalent to a crystalline drug?
A. No, it is not. Amorphous drug is exactly the opposite of a crystalline drug.
Q. Can you bring up DD-84, Mr. Broyles.
What are some of the differences between a crystalline and amorphous drug?
A. Here, we have seen this before, when the molecules are packed in a crystal, you have this very nice order of packing of molecules; whereas, in amorphous, you don't have that nice packing that we observe in crystalline.
This occurs because of the fact this packing

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order leads to --
MR. SCHEVE: Your Honor, I thought we were limited to one expert each per issue in the lawsuit. This is clearly testimony that's already been covered by a previous expert for Abraxis.
MS. KRUZE: Your Honor, he is going to be covering different issues, including the biology of these differences.
THE COURT: So, Mr. Scheve objects that this is a re-do of previous expert testimony.
MR. SCHEVE: This was used with Dr. Atwood. I only object because I am of the impression I am limited to one witness per topic.
THE COURT: You are.
MS. KRUZE: I will be happy to switch to the functional differences. This is more background.
THE COURT: We have enough background.
BY MS. KRUZE:
Q. Let's go to DD-76.
Do crystalline solids function differently than amorphous solids in pharmaceutical compositions?
A. Yes, they do. Crystalline solids dissolve a lot slower than amorphous solids. Amorphous solids dissolve a lot faster. I believe we have heard the analogy many times between cotton candy and rock candy.

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Q. Let's go to DD-77. How do these differences in dissolution affect the performance of an anticancer drug?
A. When you have a crystalline drug, the fact that the crystalline drug would circulate in the bloodstream with, it would not resolve, it would still remain as a crystal. But in the case of an amorphous drug, like Abraxane, it dissolves very fast, and that dissolved drug is able to be carried by blood into the tumor. It is really the dissolved, the molecular form of the drug that gets into the cell. And that's the one that's therapeutically most effective.
Q. Are there any scientific treatises that agree with you about this difference between crystalline and amorphous drug in anticancer pharmaceutical compositions?
A. Yes. One of the classic scientific treatises that we use in pharmaceutical science is the Remington's. It is sort of considered to be the authority in the area of pharmaceutical science.
If you look at Remington's, there is clearly a passage that differentiates amorphous and crystalline drugs that says amorphous has higher solubility and also higher bioavailability.
Q. Could you pull up DD-78, Mr. Broyles.
This is based on DX-407.
Is this the article that you were referring to?

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A. Yes. Here is a passage from Remington's, Amorphous versus Crystalline. Clearly, the crystalline state of hydration and polymorphic structure -- this is when you have different crystalline structures -- have been shown to have a significant influence on the dissolution rate. Investigators Mullin and Macek showed that this drug, novobiocin, has higher solubility, higher dissolution rate. The dissolution rate is basically a measure of how fast something dissolves than the crystalline form.
Additionally, the blood levels, this is an indicator of the bioavailability because now you are seeing how much drug is in the blood, is three to four times more in the amorphous form as compared to the crystalline form.
Q. At the bottom of the slide, that was also observed in four other compounds?
A. Yes. Here we can see that it's not a drug dependent -- it is not dependent on novobiocin. It's actually independent of the type of chemical. As long as you have these differences between crystallinity and amorphousness, it really doesn't matter which drug it is.
Q. Mr. Broyles, can you switch to DD-113. This is based on 627.
Are there any Elan experts that agree with your opinion?
A. Yes, here is one of the articles from Dr. Munson.

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Dr. Munson was an expert from Elan. He also agrees that amorphous state has a higher dissolution rate, higher solubility than does the crystalline state.
Q. Let's switch to DD-79. Is there anything in the '363 patent that teaches that the inventors believed that their invention had prolonged circulation in the blood pool?
A. Yes. There are two places in the '363 patent that mentions this issue of prolonged circulation.
It is clear that crystalline drug will have prolonged circulation in the blood pool after IV or intravenous injection in the blood. Similarly here, we see prolonged circulation in the blood pool.
Q. Are these statements consistent with an amorphous drug like Abraxane?
A. No, they are not. A drug like Abraxane or amorphous drug like Abraxane would dissolve very quickly and it would then be available at the tumor site much more rapidly.
Q. Are you familiar with Dr. Danishefsky's testimony?
A. Yes, I am. I wasn't here physically for his testimony. But I read the transcript of the testimony.
Q. Could you remind the jury who Dr. Danishefsky is?
A. Dr. Danishefsky is a professor, I believe at Memorial Sloan Kettering Institute in New York.
Q. Did you hear Dr. Danishefsky disagree with anything that you said today on these differences?

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A. No. I don't believe -- from the testimony that I read, I don't believe there was anything that he disagreed on.
Q. Are you aware of any statements made by Elan that confirm your understanding of these differences between crystallinity and amorphousness?
A. Yes. I have seen several of the reports in this trial, and in various parts by looking at all the evidence, that there has been comments about this.
Q. Mr. Broyles, can you bring up DD-80.
Dr. Amiji, can you explain to the jury what this is?
A. So here are some of those comments. We see the first one is that e-mail from -- excuse me, a memo from Glenn Portmann to Dr. Gary Liversidge. He clearly says, Amorphous state has been known to have enhanced bioavailability. This is the measure of how much drug that's going to be available at the disease site.
Distinct from particle size considerations, as you are well-aware, and then it says, Amorphous form usually possesses higher thermodynamic energy. This just means that they are in the unstable state than the crystalline counterparts resulting in solubilities and dissolution rates of much higher magnitude.
They also go on to say, in the '684 patent file

IN THE UNITED STATES DISTRICT COURT

IN AND FOR THE DISTRICT OF DELAWARE

- - -

ELAN PHARMA INTERNATIONAL LIMITED,	:	Civil Action
Plaintiff,	:	
v.	:	
ABRAXIS BIOSCIENCE INC.,	:	
Defendant.	:	No. 06-438-GMS

- - -

Wilmington, Delaware
Tuesday, June 10, 2008
8:45 a.m.
SEVENTH DAY OF TRIAL

- - -

BEFORE: HONORABLE GREGORY M. SLEET, Chief Judge, and a Jury

APPEARANCES:

JOHN G. DAY, ESQ.
Ashby & Geddes
-and-
STEPHEN SCHEVE, ESQ.,
LINDA M. GLOVER, ESQ.,
JEFFREY SULLIVAN, ESQ.,
ROBERT RIDDLE, ESQ., and
PAUL FEHLNER, ESQ.
Baker Botts LLP
(Houston, TX)
-and-
GREGORY BOKAR, ESQ.
Counsel - Elan Drug Delivery

Counsel for Plaintiff

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Felner at the University of Davis, we were able to cure diabetic dogs who were pets of the veterinary school, with a single injection.

And we went on in 1993 then to do the country's first encapsulated islet cell transplant at UCLA and at St. Vincent's Medical Center. And we published this in Lancet in 1993.

So to me this was a --

BY MR. JACOBS:

Q. I think the battery may be going.

(Pause.)

Q. Let's just catch the jury up with what you are describing right now.

A. Okay. So we conceived of, developed, successfully developed a cross-linked capsule, which acted like a tea bag, placed living cells in, kept the cells alive. Placed them into the abdominal cavity with a single injection into a patient, literally a ten-minute procedure. Now the patient could have insulin being secreted and the body cells, rejection cells, could not reach the cells to reject it.

This was the country's first transplant ever of an encapsulated islet cell.

So this, clearly, was to me an exciting moment. And the idea was, now, the problem and the frustration was

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there were not enough human pancreases to treat a million diabetics.

Q. The reason that was important is because the pancreases were the source of the islet cells?

A. That's correct, sir.

So the next innovation was could we identify within this pancreas the stem cell. So by 1991 to 1992, I applied to NASA, and NASA had, as I said, I was working earlier with NASA, a tissue engineering division in which they wanted to create tissue for Mars and to grow tissue.

So I applied for a grant to NASA and received a two-million-dollar grant from NASA and started working on stem cells, in which we could actually grow and proliferate these cells.

So you could take one cell, as a stem cell and grow them. What was exciting about that, one then could then have an unlimited supply of cells for mankind.

So these cells then actually started to make insulin and died. It made insulin, and died.

As these cells grew, they grew rapidly. They made insulin, and they died. This was a puzzle to us. This was the mystery that we couldn't solve.

It turns out that at the same time, NASA was also taking albumin up into the Space Shuttle, because albumin was an important nutrient for living cells, to

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understand the chemical structure of albumin.

Then, truly, the light bulb went on. It was, maybe these cells were dying because they needed a blood supply. And if, indeed, albumin was a very important nutrient that these cells were not getting albumin, and it turned out exactly the case, that in order for these cells as they grow, they needed to feed. In order for them to feed, they needed albumin, because albumin brought with it vitamins, fatty acids, nutrients. And we then grew these living cells with blood vessels. And they survived.

So --

Q. At this point the purpose of feeding these cells the albumin is that you want these cells to flourish. You want them to grow so that they can secrete --

A. Insulin.

So when you looked at this, we looked at this, and then, if you looked at this itself, while on the one hand we are trying to treat diabetes, this actually looked to me, and I say remember I was working on cancer as well, what I would see when I would see a patient with cancer. That a patient with a cancer had cells that would proliferate and grow. That's how they would spread. And around all the cancer tissue, there would be a huge number of blood vessels. We think we now know its angiogenesis.

It dawned upon me, that is what was happening

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with cancer biology. As the cancer was spreading, the way that cancer was spreading, it was actually drawing to itself albumin to feed itself to the detriment of the rest of the body.

That's why, I believe, we lose weight when we have cancer. You can have the smallest cancer and lose a huge amount of weight because the tumor had a mechanism of extracting all the nutrients to itself. And the key nutrient it was extracting was albumin.

So from that, then, came the concept, if, indeed, that were the case, we would be able to then take this albumin and cross-link it, just like my cross-linking, and place within this albumin an amorphous drug, any drug. And now, if that were inside and injected inside the blood vessel, the tumor would, by its natural biology, rapidly pull it out of the bloodstream to itself.

To me, that to me was just an epiphany in the sense that the dogma at that time and the dogma still is, frankly, that to give chemotherapy, you just want it in the blood.

But to me, the dogma was, you didn't want it in the blood. You wanted it out of the blood. You wanted it rapidly into the tumor cell. How would you get it there? It turns out, the tumor cell was actually asking for it by putting albumin in.

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So then if you then could make an albumin nanoparticle, cross-link it, and put inside an amorphous paclitaxel, which would actually dissolve or make rapidly bioavailable, if you then take this albumin and you actually cross-linked it, and made this amorphous, you would then have a particle that could forever change chemotherapy.

So by '92, we started developing actually this cross-linked, we started developing this amorphous form. And by 1992, I got invited by the NCI, who had actually invented the taxol --

Q. Th CIS is?

A. The National Cancer Institute.

Q. A government funded, or government organization?

A. It is the government organization that is charged with discovering cancer drugs for this country.

They had identified taxol since 1970s. The problem is, taxol, the only way they could deliver Taxol was in a toxic solvent Cremophor. And I saw yet another opportunity to better treat the patient without the toxicities, whether with rejection drugs or Cremophor, by putting it into a nanoparticle without Cremophor and more importantly drive it to the tumor.

So they introduced us to Bristol Myers, and we created this compound called Capxol. Why was it Capxol? Because it was encapsulated taxol. That was our first

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prototype that we handed to Bristol Myers in 1995.

So if you look, then, at -- sorry to put my back to you. But 1986, whole organ pancreas transplant. 1991 to -- 1989 to 1993 doing islet cell transplant.

And by 1991, 1992, understanding the albumin, and creating the first prototype in 1992. And handing the first molecule to Bristol Myers in 1995.

While this looks like almost a decade of work, of discontinued thought, it was absolutely connected, that is exactly how science works, you build on your knowledge from one to the other.

By this time, we really had a wonderful team working with NASA. We had about 50 scientists here. And around this time is when Neil Desai joined our group. And we evolved that concept down to that concept.

So at the end of the day, this then became Abraxane. So this is how diabetes converted to Abraxane.

Q. Thank you, Dr. Soon-Shiong.

I would like to ask you now to explain to the jury your understanding of how Abraxane actually works in the body?

A. Your Honor, I have got a videotape. The way I would like to do it is present the videotape. May I go up to that?

THE COURT: You may.

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MR. SCHEVE: Your Honor, has a copy been provided for us?

THE COURT: I assume that this was all --

MR. JACOBS: Absolutely, Your Honor.

MR. SCHEVE: I guess it was. My fault.

THE COURT: All right.

THE WITNESS: What I would like to do, this is a videotape with, actually, not prepared for this case. We had prepared this videotape for the scientists and the doctors at the -- the cancer doctors, because we wanted to explain this new science. This is a total new science with regard to chemotherapy.

I think we created this tape around 2003-2004, with the idea that this would be an explanatory tool.

So for the purpose of this case -- obviously, that tape was fairly long -- we reduced it to maybe one-fourth of that. All we have done is just put labels, just so that would be clear.

What I would like to do is take you through --

BY MR. JACOBS:

Q. Before you do that, I think it might help if we dim the lights some.

A. What I would like to do is take you through this videotape first rapidly so you can see where you are inside the body system. Then replay it slowly to explain how I

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believe Abraxane works.

So if we could start the tape. What we have here is a solid tumor. As I said, within cancer, one of the things that you see, blood vessels feeding the tumor. We go now rapidly into the blood vessel, as you inject the drug. Now you are inside the blood vessel. And here is the red cell and here is our nanoparticles inside the blood vessel.

I will come back to this as we go through it in detail. This is the structure of the nanoparticle. It's amorphous paclitaxel. And in order to contain amorphous paclitaxel, it needs to be cross-linked. This amorphous paclitaxel dissolves once injected. Now you are inside the blood.

The question you want to get from there to the other side, this is the blood vessel and the tumor is on the other side.

And the amorphous paclitaxel dissolves inside the blood vessel. The question is, then, how does it get to the other side? You are now inside the blood vessel. I will speak a little to this receptor activates. And it creates an elevator, a physical elevator, within the blood vessel wall itself. That's the beauty of this science. This is what the tumor does to extract albumin to itself, so it gets to the tumor .

Then the albumin gets into the tumor and feeds

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the tumor. However, if we put a drug inside this albumin, it will kill the tumor.

So, in a rapid run-through, that was how Abraxane worked. If I may now take you through this a little slower and start it again, Your Honor.

So, as I said, one of the ideas is, it's very clear that when you have the tumor, the tumor grows blood vessels around itself. It's called angiogenesis. Why is it doing this? It's doing this to feed itself.

So the idea came to us, rather than starve the tumor, which by the way, Avestin and Genentech and other mechanisms were trying to do, kill these blood vessels. Another way to do it is to feed the tumor, but feed the tumor poison. Why this to us was important was, to me, this was ubiquitous to all tumor types, meaning any tumor, whether breast, lung, pancreatic, would actually have this mechanism.

You are then now inside the blood vessel. Now, when you are inside the blood vessel, as you inject this particle, it has to be small enough, the nanoparticle has to be small enough for many reasons. One, small enough so it can get through a sterile filter. A sterile filter is 220 nanometers. So it needs to be below that so you can actually filter this and you can inject this into human beings, because you cannot sterilize albumin. If you

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sterilize albumin you would actually destroy it. The only way to sterilize it is put it through a filter and remove any contaminants.

The other reason you need it to be small enough, so you can get it into the smallest blood vessel. This is a single red blood cell inside the blood vessel.

As you can see, a nanoparticle is 1/80th the size of a single red blood cell.

There is a tremendous challenge here. You needed to make it small. You needed to make it stable. And you needed to keep it so that you can actually filter it.

The particle is injected, and let me now explain to you the structure of this particle.

In order for us to accomplish that goal of making it small enough, and yet when it gets in to rapidly dissolve, you needed to make the paclitaxel amorphous.

As we described in our papers, that in order to make this dissolve, it needs to be amorphous, like cotton candy. If you made it crystalline, you would actually design it not to dissolve, like rock candy, just to stay there.

So we had two different paths. We wanted ours to dissolve rapidly and not stay inside the blood. So, therefore, ours had to be amorphous, not crystalline. But because it's amorphous, you had to contain it. And the only

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way to contain it, you could put it with the albumin, but you need to cross-link the albumin.

So this was the albumin cross-linked, like a weave.

And this contained the nanoparticle in its cross-linked form.

But then the albumin would actually be able to now see these receptors.

So once injected, it would then go and rapidly dissolve. So here is the amorphous nature, taking in space, now you have paclitaxel and albumin together.

Now the question is, how does it get out?

Well, what it does, it now seeks -- how does it go from here magically to here? We needed to cross this physical blood vessel wall. The way it does that is it seeks out on the inside of the blood vessel a receptor. If you could hold this right here. Now we are inside the blood vessel. It is dissolved. It turns out that this thing called a gp60 receptor is overexpressed in cancer. By that we mean, as these cancer cells grow and these blood vessels grow around it, these blood vessels are a little abnormal.

What they do, they actually express all these little receptors, so it can pull albumin to itself.

What is exciting to us, then, we have now a tumor-selective mechanism. It goes to the tumor, not to the

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rest of the body, so we can reduce the toxicity, increase the tumor concentration, increase the efficacy, by virtue of this interaction between this albumin and this receptor that the tumor overexpresses.

So what happens when it actually locks in here? It then activates this key called caviolin-1. Once it activates this key, it opens the elevator door, and in comes all the albumin molecules, and it goes through. It literally physically creates this hole called a caviolin. It literally creates this hole, and physically transports it to the other side.

This occurs literally within minutes of injection. So to us, this was what I considered the transforming event for chemotherapy, because now, rather than the dogma of keeping it in the blood, the transforming event is to get it out of the blood because if you want to kill a cancer, you don't want it there. You want it here.

And you want it here rapidly.

We achieved that through this mechanism. Now it gets to the tumor cells, and when it gets to the tumor cells, this fatty acid membrane takes this albumin, unlocks the drug, takes it into its nucleus, and shrinks the tumor rapidly.

So that is the mechanism of Abraxane. And by supplying the tumor with albumin-bound nanoparticles, this

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is what we had approved first in breast cancer.
BY MR. JACOBS:
Q. Thank you, Dr. Soon-Shiong. You can resume your seat.
(Witness resumes stand.)
Q. I need to bring you now to the specific patent issues in this case. You became aware of Elan's '363 patent sometime toward the end of August 1996. You followed Elan's technology over the years.
Do you believe that Abraxane infringes the '363 patent?
MR. SCHEVE: Objection, Your Honor, to there being no foundation for this witness to express an opinion without a foundation about even the claim construction terms that Your Honor has provided.
THE COURT: Please, Mr. Jacobs.
BY MR. JACOBS:
Q. Did you form a judgment at that time, did you form an opinion yourself, when you have seen the '363 patent, whether you were infringing that patent?
MR. SCHEVE: Same objection, Your Honor.
THE COURT: I don't have to tell you how to do this, Mr. Jacobs, you have to establish that he has seen the '363 patent and he is familiar with the claims.
BY MR. SCHEVE:
Q. Did you see the '363 patent?

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A. Yes.
Q. Did you form a judgment about whether Abraxane and whether Abraxis would be infringing it?
A. Yes.
Q. What was that judgment?
A. I was very clear, not at all.
Q. Why is that? What was the basis for that belief?
A. Well, firstly, our design was totally opposite, clearly, I think, as I tried to express. Maybe it would be easier if I may just go to two pictures on that to express that, if I may.
(Witness steps down from stand.)
THE WITNESS: Sorry, Your Honor. Our design was such that we wanted our drug rapidly out of this, rapidly out of the system.
So from a purpose or focus of our design is we wanted our drug rapidly out. My understanding of Elan's strategy is they wanted to prolong the drug inside the vascular system, 180-degree opposite strategy.
In order for us, however, to get our drug out rapidly, the only way we could get out rapidly is by designing an amorphous system. The only way they could get it to stay inside the drug for a long time is to keep it crystalline. So the crystalline nature and the amorphous nature achieves two direct opposite purposes. And if I may,

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then, show it.
So we wanted it amorphous so we can get it out. They wanted it crystalline so they could keep it in. And in order for us to make it, keep it amorphous, we needed to keep it cross-linked. They had theirs non-cross-linked.
So if one is amorphous, the other is crystalline, the other is cross-linked, another one is non-cross-linked, to me, it was very clear. These were two different paths, two different missions.
BY MR. JACOBS:
Q. Thank you.
(Witness resumes stand.)
Q. You mentioned in the first part of your remarks the contact you had with Bristol Myers Squibb in 1995. Can you describe that contact and what came of it?
A. Yes. The NCI introduced us to Bristol Myers Squibb in 1992-1993 time frame. They gave us paclitaxel. It was a rare opportunity for us to get our hands on the paclitaxel. We made the nanoparticle, provided it to Bristol Myers. They tested it. And they found it to be active. And then turned around and said, unfortunately, they didn't believe we could manufacture this.
So nobody ever manufactured a human protein nanoparticle in large scale. We are talking about kilograms, thousands of grams of paclitaxel and nobody had

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ever done this on a commercial scale. And they declined.
Q. How did that affect your plans or thinking about the development of Abraxane?
A. It was a very, very difficult time for us, because we now had 50 people. And the choice to us was, do we drop this drug? And we did not have expertise in scale-up or large scale-up as a pharmaceutical company.
So we had to make a choice, and I took the choice of taking the risk at that point, and approaching a company in Chicago, owned by the Japanese, that had the largest plant in the United States that did all anticancer drugs, a large number of FDA approval for injectables. And they had this plant in Chicago with 500 people. But they were losing a million and a half a month, truly losing money.
They wanted to sell this plant. They put it up for sale with JP Morgan. And I had to convince investors to say, please buy this plant, it's losing one and a half million a month, because it is important for me to figure out a way to scale this and manufacture this because we cannot let this go.
So we went ahead and, this is what became APP.
Q. And APP was a predecessor company to Abraxis?
A. Well, it was the other way around. Abraxis owned the technology. We created within Abraxis a subsidiary called

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Q. Who doctored the images to take out the smaller ones here that were aggregating with the larger images? Who doctored that photo?
A. This was probably done by the IT department, as I said, to colorize it.
Q. IT at what place, sir, which entity?
A. I don't remember. You said who doctored it, I don't know. You are trying to connotate a negative -- this is a cartoon. Nobody doctored it. They actually colorized it, as I told you, to make it a cartoon. They may have not liked the fact, to make it clear -- I don't know who -- I can't recall.
Q. A cartoon.
Let's go back to Dr. Grinstaff's publication.
Dr. Grinstaff and Dr. Suslick. Now, sir, isn't it true that this image that they have designed as Figure 1 actually is a scanning electron micrograph of a dodecane filled proteinaceous microcapsule with an average particle size of 2.5 microns, and that this microcapsule was using bovine serum albumin?
A. That's correct.
Q. There isn't even a drug in those microcapsules, is there, sir?
A. That's correct.
Q. Now --

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MR. JACOBS: Your Honor, I object to this whole line of questioning.
THE COURT: Okay.
(The following took place at sidebar.)
THE COURT: What is the basis of the objection?
MR. JACOBS: Relevance. Prejudice.
THE COURT: Well, relevance first.
MR. JACOBS: The question is, in this lawsuit, whether Abraxane is amorphous or --
THE COURT: Let me say, before you go forward, you are going to this witness' credibility.
MR. SCHEVE: I have heard this story. This was my idea, and we are so different. He lifted this stuff from a fellow one-year doctor before Dr. Desai graduated from school. It is credibility.
THE COURT: Your 403 argument -- it is prejudicial. The question is, is it unfair?
MR. JACOBS: Yes, I think this is unfair. We got in an exhibit last night at 10:30 p.m.
THE COURT: You should have raised that earlier.
MR. JACOBS: Your Honor, we should have raised half of the stuff that is going on between the parties.
THE COURT: I am sure both of you have not done that, and wisely.
MR. JACOBS: Now he is using this exhibit with

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this witness, our CEO, and saying he doctored the photograph. This is character assassination.
MR. SCHEVE: He said probably his IT department did it.
THE COURT: Actually, the last word he said was he didn't know, to be fair.
MR. SCHEVE: I am moving on to a new topic.
THE COURT: Let's move on.
(End of sidebar conference.)
BY MR. SCHEVE:
Q. Doctor, there has been some testimony about a product that is marketed that utilizes the NanoCrystal technology Rapamune.
Are you trying to apply your NAB platform to this product?
A. The Rapamune that you are talking about, there is the oral version of Rapamune taken by mouth. To this day, there is no injectable version of Rapamune at all, because of its insoluble. So the answer is yes.
So we have actually now encapsulated, in our cross-link, a form of Rapamune. And we are about to -- we actually received FDA approval to initiate Phase 1 trials.
Q. If Abraxis is able to somehow challenge these patents and somehow get them declared invalid, then you wouldn't have to confront them as you move forward with your efforts

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to develop Rapamune and, indeed, other products, like camptothecin, and others to which the '363 technology has been applied. Correct?
A. Well, it's not correct, because we are not confronting them anyway. We don't believe this has anything to do with our technology or our patents.
Q. You may believe that, sir, but I am just asking the question about, if the patents aren't there, then you don't have to worry about them at all. Correct?
A. Well, if patents aren't there, you don't have worry about them at all, that is correct.
Q. Now, sir, does the package insert for Abraxane advise physicians to reconstitute and administer it to patients?
A. Yes.
Q. And then did Abraxis obtain or rely upon the opinion of qualified patent counsel as to whether the nanoparticles in Abraxane would infringe Elan's '363 patent before making, offering to sell and/or selling Abraxane?
A. We were fully aware that we are two different particles. We had collaborations, discussions, in which we knew we had two different particles. One was crystalline, the other one was amorphous. One is cross-linked, one is non-cross-linked. One wanted to stay in the blood, the other one wanted to get out of the blood.
When we looked at patents of the '363, it states

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within the body of the patents, we are crystalline, not amorphous. In the body of the patents, of the Elan patents, it says we are crystalline, not amorphous. In order for us to become amorphous, you need to do a different method of manufacture, solvent precipitation, which we do. In order to become crystalline, we need to do a different method of manufacture, grinding, which they do.

So if they told the Patent Office that they were different, we told the Patent Office we were different. We scientifically knew we were different. We were almost professional colleagues. We were different.

So there was no issue from our perspective.

MR. SCHEVE: Your Honor, may I move to strike the answer? I asked him whether they obtained an opinion of counsel. As you recall in the pretrial conference, there was an order instructing Mr. Jacobs to --

MR. JACOBS: Your Honor, objection. Sidebar.

THE COURT: You would like a sidebar, Mr. Jacobs?

MR. JACOBS: Please, Your Honor.

THE COURT: Let's calm down.

(The following took place at sidebar.)

MR. JACOBS: Mr. Scheve should not be talking about orders and directions in front of the jury. That is very much not the way Your Honor runs the courtroom.

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THE COURT: That is true.

MR. JACOBS: Number 2, we were working out a stipulation on this, all of a sudden Elan went silent on us. I asked for the words back in the draft. They went silent.

Number 3, we will stipulate to exactly the way Mr. Scheve said it. If that will move us on.

MR. SCHEVE: The background on that, Your Honor, we submitted to them three days ago -- we never got the amended interrogatory answers as you ordered Mr. Jacobs to do. So we put together the proposed language, sent it to them three days ago. I can show Your Honor -- I know you don't want to get involved in those details.

THE COURT: We don't have time.

MR. SCHEVE: I am just asking, did they get an opinion? And he says, we looked at patents, they looked at patents. The patents are different. I want to know, did you get an opinion or not? If they want to stipulate it, I'm able to read it into the record, I am done with this witness.

Can I get these two stipulations here that they didn't get an opinion of a qualified patent counsel as to whether the nanoparticles would infringe Elan's '363 before making, offering to sell and/or selling and a separate one that they obtained or relied upon an opinion of qualified patent counsel as to whether the '363 patent was valid

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and/or enforceable before making, offering to sale, and/or selling? Will I get those two stipulations?

MR. JACOBS: So stipulated.

MR. SCHEVE: I am finished with the witness.

THE COURT: Do you object to them being announced by Mr. Scheve?

MR. JACOBS: No.

MR. SCHEVE: Your Honor, would you like me to dismiss the witness and then read the stipulation, or do it while he is on the stand?

THE COURT: While he is on the stand or after?

MR. JACOBS: After.

(End of sidebar conference.)

THE COURT: Doctor, I am advised we have come to the end of the cross-examination. You are excused.

MR. JACOBS: Your Honor, could we have one minute to confer on redirect?

THE COURT: I am sorry.

MR. SCHEVE: May I read the stipulation?

THE COURT: We will announce it.

MR. SCHEVE: Abraxis has stipulated, meaning that they agree, that they did not obtain or rely upon an opinion of qualified patent counsel as to whether the nanoparticles in Abraxane would infringe Elan's '363 patent for making, offering to sell, and/or selling Abraxane.

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In addition, they stipulate or agree that Abraxis did not obtain or rely upon an opinion of qualified patent counsel as to whether the '363 patent was valid and/or enforceable before making, offering to sell, and/or selling Abraxane.

THE COURT: That is another one of those rare occasions, ladies and gentlemen; there is an agreement there.

REDIRECT EXAMINATION

BY MR. JACOBS:

Q. Dr. Soon-Shiong, I only have one more question for you on what we call redirect. Why not?

A. Because we absolutely felt very clear from our perspective, we felt we knew it, we felt they knew it. It wasn't an issue for us, because we were developing two different pathways. We had an amorphous version. They had a crystalline version. We had a cross-linked version. They had a non-cross-linked version.

This may be interesting, it's sort of interesting. The same patent examiner was examining both patents at the same time. It's just an interesting, I suppose, coincidence or maybe not, because this is the examiner in this field at the U.S. Patent Office, Thurmond Page, was the same examiner examining our patents, examining their patents. And the Patent Office felt it was different.

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- - -

Wilmington, Delaware
Wednesday, June 11, 2008
9:40 a.m.
EIGHTH DAY OF TRIAL

- - -

BEFORE: HONORABLE GREGORY M. SLEET, Chief Judge, and a Jury

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JOHN G. DAY, ESQ.
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(San Francisco, CA)

Counsel for Defendant

- - -

THE COURT: Good morning. Please take your seats.

We will take the JMOLs first. Other than to -- I will indicate that I have had a chance to, albeit an abbreviated opportunity to review your various positions, I have considered them. Contrary to what I announced yesterday, I will act on at least a couple of things on each side.

Largely, for the reasons articulated in various submissions, I'm not going to have time to prepare a more articulate analysis than that. I will tell you that I am going to do the following.

As to plaintiff's various JMOLs -- and I gather counsel will recognize that I am not interested in hearing argument. This is, I think, a housekeeping matter more than

anything, that Abraxis in one of its headings at Page 8 of its motion, points out that Abraxis has failed to put on certain evidence to support certain defenses. I don't think this is contested. If it is -- well, we'll see.

Obviousness, obvious type double patenting, indefiniteness, I think are the three. I think you would concede those, wouldn't you?

MR. JACOBS: Yes, Your Honor.

THE COURT: Okay. So those are out. In other words, the motion is granted.

The Court agrees with Elan that the reverse doctrine of equivalents is not supported by the law and the facts of record in this case. The Court agrees with Elan that Abraxis's defense of unclean hands is not properly submitted to the jury in this case.

Unless you can tell me why I should not so find, Mr. Jacobs, I don't see any other basis for this jury, any other damage theory for this jury to consider other than that which has been offered by Elan.

MR. JACOBS: On the theory, we agree, Your Honor.

THE COURT: So that's granted. Those are all granted.

The others are denied. Not reserved. Denied. Of course, they can be raised again.

I am going to agree, Mr. Scheve, with Mr. Jacobs that Abraxane does not infringe under the doctrine of equivalents, nor has Abraxis induced infringement or contributorily infringed. I have already decided on the reverse doctrine of equivalents issue in Elan's favor.

The remaining motions are denied.

That's JMOLs.

Any questions about any of those rulings? Do we understand where we are?

MR. SCHEVE: I am putting that question to my co-counsel, Your Honor, just to make sure that I caught it correctly.

MR. SULLIVAN: Your Honor, I have advised Mr. Scheve, and I hope correctly, that Elan will not be permitted to put on a case of doctrine of equivalents infringement.

THE COURT: That's right.

MR. SULLIVAN: Thank you, sir.

THE COURT: All right. All right. Let's take a few moments and go through these remaining disputes on the parties' proposed final general instructions.

So the first one I have is at Page 9. The parties and their contentions. I see that Abraxis objects to the bracketed language as unnecessary, argumentative, redundant, confusing.

MR. JACOBS: Your Honor, we resolved that, we think.

THE COURT: Fine.

MR. JACOBS: That is what I am told.

THE COURT: That language is going to remain?

MR. WALTERS: I apologize, Your Honor. This is an agreement reached just as we walked in the door. We have substitute language for that instruction that the parties have agreed on.

THE COURT: I would like to see it.

MR. WALTERS: It was in one of your packets. It may not have made it up there. I apologize for that.

THE COURT: It's not in any of the packets I have. I mean, I have got enough packets that it's an amazing thing that I think I have come out with the right materials. So if you want to give me the language, I will look at it.

I think in the fourth line, there is a typo issue to be referred to as paclitaxel or taxol, not "a."

As best I can tell, the change from the version that I have is in the last sentence. The version I have been provided just a moment ago reads, the last sentence, the case also involves certain questions regarding the validity and enforceability of Elan's '363 and '025 patents.

The version submitted this morning earlier

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the facts," and that offended you, please hold me responsible. Don't hold my client responsible. If I offended you in any way, I apologize for that.

This case can really be boiled down to two issues. I am going to get to what the evidence is. At the risk of going over territory you have already heard, let's just go over a few of the slides that I showed you during opening to sort of give us some bearing on what the evidence shows in this case.

You have heard about this compound called Taxol. It contains this drug that was originally derived from a yew tree. When I sit down this weekend to talk with my friends, among the things I am going to say is I met Sam Danishefsky. If there wasn't Sam Danishefsky, there wouldn't be an Abraxane. There wouldn't be the nanoparticulate formulations of paclitaxel. And we all got to meet him in this case.

He was the person who was able to synthesize this so that they could make paclitaxel, not kill all those yew trees up in the Northwest. And that allowed for the development of different formulations. The original formulation, called Taxol, which is made by Bristol Myers Squibb, is toxic, as you have heard in this case, because it contains solvents. Cremophor is a very, very toxic solvent. It is made from castor beans. It is actually more toxic

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than Taxol itself. It requires premedication with antihistamines, and it limits the maximum-tolerated dose.

Nanoparticles, you have heard testimony, why are they beneficial when smaller means more exposed surface area? More exposed surface area means better dissolution rate. Better dissolution rate means we hope that you are going to get more of the chemical or drug into your system so that it can do what's hoped for.

What was Elan's solution? What was Dr. Liversidge's and Dr. Liversidge's solution? Reduce particle size, the stabilizer adsorbed onto the surface of the particle, to prevent clumping. We showed you an example, when we poured that stuff in the water and that stuff clumped up, that's what poorly soluble molecules do.

The hope was here that they could separate these particles in such a way that there wouldn't have to be these terrible solvents which create all sorts of problems and require premedication.

The proteins are adsorbed onto the surface of the crystalline medicament, and they function in two different ways. One is through electrical charges, and others, as Dr. Liversidge described, is sort of failing arms prevent these particles from coming together and clumping.

Adsorbed, what does it mean? It means physical contact with the surface. No penetration of the surface and

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no chemical reaction.

Who were the people behind this? Dr. Gary Liversidge, Dr. Elaine Liversidge, you have heard about their accomplishments. Dr. Liversidge told you the thing that means the most to him is every day 1.5 million people take a compound that contains one of his inventions.

The invention claimed are particles. It's very important, ladies and gentlemen, that when you go back to do your deliberation you understand, we are not claiming that Abraxane as a product infringes. We are claiming that Abraxane contains particles and that those particles infringe.

You heard testimony in this case that there are 61 trillion particles in a bottle of Abraxane. 61 trillion is over 10,000 times the number of human beings living on this earth. And the question is, among those 61 trillion, are there more than one particle -- are there particles that embody the invention of the '363 patent and therefore infringe it?

So it's particles consisting essentially of crystalline medicament and a surface modifier essentially free of intermolecular cross-linkages. And as Judge Sleet has instructed you, the first question is below 1,000 nanometers, and the other claim is below 300 nanometers in size.

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Now, there is a problem with putting nanoparticles in the human body, because bodies react to it. It's called an anaphylactic reaction. It can be deadly, as you heard testimony about.

My clients also patented the '025 patent, which was a method of intravenous administration to a mammal within a certain period of time, according to a certain formula for administration. My client is not before you claiming that Abraxis infringed that patent. Nonetheless, Abraxis wants you to declare this patent to be invalid.

It's not unusual in patent cases, if you have been accused of infringement, to have as an immediate response, well, your patent is no good. Therefore, I shouldn't be held liable.

When you checked in this building, you probably were asked to check your cellphone. But you weren't asked to check your common sense. As we go through and I go through the evidence with you, I am going to ask you to draw on your common sense, your common experiences in life, look at this evidence. I am going to ask you to put up experts side by side and witnesses side by side. Use your common sense, draw inferences, and reach the conclusions that we believe the evidence shows.

You will be happy to know that I am then going to go to materials up on the screen and get rid of these

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third, which the computer did analysis on, if I could go to the next slide, please. Dr. Munson -- this was the freeze-dried sample -- after he subtracted out these other two human beings, there was a third one there. Eight percent of the total paclitaxel within those vials of Abraxane are crystalline. Eight percent of the total. Very important number to remember. Eight percent of the total, because the issue here isn't in this case is there a certain percentage of the total. The question is are there nanoparticles, more than two, amongst 61 trillion, that are, as Judge Sleet has instructed you, that are themselves entirely crystalline. We know that eight percent of the total was crystalline, based on this definitive study. We know that in the air-dried sample, it was seven percent.

Dr. Munson testified to you that gave him some confidence that there wasn't any sort of artifacts or artifactual data that was being seen.

Single bottle of Abraxane contains over 60 trillion particles. If eight percent of those particles contain crystallinity, then over 4.8 trillion particles in a vial have a crystalline content.

If only four percent, then 2.4 trillion particles contain crystalline medicament.

The question that you have to decide, based on the evidence that you have seen here is, of those 60-plus

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trillion particles, are there two or more that are particles wherein the paclitaxel is crystalline as has been construed for you by the Judge in the instructions you have been given?

Again, the law isn't, well, it doesn't infringe if it's just a few. Personally, I don't think 2.4 transitional is just a few. If it contains particles and they meet the claim limitation, then that's infringement. That's what the law is. The law allows my client and anybody else who has got a patent to seek to prevent others from using that invention. And we seek a license for the fact that, notwithstanding their best efforts, there are crystalline particles therein.

Now, Dr. Munson then offered the following opinions. Abraxane contains particles consisting essentially of 99 to 10 percent by weight of a crystalline medicament useful in treating cancer. Abraxane contains particles that meet the crystallinity claim limitation as defined by Judge Sleet.

What else do we know on this crystallinity issue?

We know that the starting material, starting paclitaxel is a white to off-white crystalline material. They start with crystals.

What else did we know? You heard Dr. Atwood,

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their expert, comment on this. In the published literature since 1955, one of the most remarkable facts revealed by x-ray science is the extreme rarity of a truly amorphous state. The extreme rarity of a truly amorphous state.

You begin to add the evidence up. DSC, this is not a hundred-percent amorphous. It is the fingerprint. That testimony is not contradicted by anybody. Dr. Berkland looks at the cryo-TEM. He sees some angles and some edges. He says it's suggestive of crystallinity. We go to the definitive test, ssNMR. We know that eight percent of the total paclitaxel contained is crystalline. And Dr. Munson gave you his opinion, there are particles in there that meet the claim limitation.

Now, you heard evidence in this case about, well, wait a minute, there is this test called x-ray powder diffraction, and it shows that this is amorphous.

You saw this chart. When you go back to deliberate, I am going to ask you please to look at this real close, because the evidence really didn't focus on -- or I should say the testimony. These lines all look about the same. What's important is that you look at this scale. You remember during the testimony that this bottom line is crystalline paclitaxel, particles up to 500 microns. That is 3500 times bigger than the nanoparticles within Abraxane. 3500 times bigger.

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The reason I say look at this scale, because the largest peak goes up to 7,000, just under 7,000.

You will also recall this lineup here. This is what their expert pointed to, Dr. Atwood, which was a physical mixture. They just took that raw paclitaxel, somewhere between 50 and 500 microns, up to 3500 times larger than the particles in Abraxane. And they just added albumin to it. That's all they did. They didn't reduce it in size. They just added albumin to it.

Look at the difference in the shape by just adding albumin to particles that big.

What we provide to you now is, if you chart these, no reduction in size, all you do is add albumin to these large particles. And look what happens to the line. Look at the difference in the scale. The highest peak here, when you look at it, is about 600. You remember, this one that went up to close to 6800. Look where the peak is here. Why? Why? What's the effect of albumin? What things affect x-ray powder diffraction?

Again, I am going to ask you to consider the testimony of the woman that I asked the Texas phrase to -- I shouldn't say that because I grew up someplace other than Texas. This was Katherine Melody. I said, Ms. Melody, you don't have a dog in this hunt, do you? She didn't know quite what I meant. I was trying to ask her, you are not