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(54) Titre : STABILISATION D'AGENTS ACTIFS PAR FORMULATION SOUS FORME NANOPARTICULAIRE
(54) Title: STABILIZATION OF ACTIVE AGENTS BY FORMULATION INTO NANOPARTICULATE FORM

(57) **Abrégé/Abstract:**

Methods for stabilizing active agents, particularly pharmaceutical agents, are described. The method comprises forming active agents into a nanoparticulate composition comprising the active agent and at least one surface stabilizer. The component active agent exhibits chemical stability, even following exposure to, for example, a prolonged storage period, an elevated temperature, light, radiation, radiation causing photolysis, non-physiological pH, enzymes or other catalysts, water or other solvent molecules, oxidizing agents or other free radicals, or freezing.



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(54) Title: STABILIZATION OF ACTIVE AGENTS BY FORMULATION INTO NANOPARTICULATE FORM

(57) Abstract: Methods for stabilizing active agents, particularly pharmaceutical agents, are described. The method comprises forming active agents into a nanoparticulate composition comprising the active agent and at least one surface stabilizer. The component active agent exhibits chemical stability, even following exposure to, for example, a prolonged storage period, an elevated temperature, light, radiation, radiation causing photolysis, non-physiological pH, enzymes or other catalysts, water or other solvent molecules, oxidizing agents or other free radicals, or freezing.

STABILIZATION OF ACTIVE AGENTS BY FORMULATION INTO NANOPARTICULATE FORM

FIELD OF THE INVENTION

The present invention is directed to methods for stabilizing active agents, particularly pharmaceutical agents, comprising formulating a at least one active agent, which is unstable under one or more environmental conditions, into a nanoparticulate composition. The nanoparticulate active agent is stable under the same environmental conditions under which the active agent is unstable prior to formulating the agent into a nanoparticulate composition. The nanoparticulate composition comprises an active agent and one or more surface stabilizers adhered to the surface of the active agent.

BACKGROUND OF THE INVENTION**A. Summary of Instability and/or Degradation of Active Agents**

Active agents are subject to degradation. Such decomposition or degradation may be due to hydrolysis, oxidation, isomerization, epimerization, or photolysis. The rate of degradation or decomposition varies considerably depending on the structural, physical, and chemical nature of the active agent. The rate of decomposition is also often significantly affected by numerous environmental factors, including temperature, light, radiation, enzyme or other catalysts, pH and ionic strength of the solution, solvent type, and buffer species.

Chemical instability due to degradation or decomposition is highly undesirable for several reasons. For example, when a chemical compound is a pharmaceutical agent, degradation decreases its efficiency and shortens its effective shelf life. Moreover, the decrease in the content of the active ingredient in a pharmaceutical preparation renders the calculation of an effective dosage unpredictable and difficult. Furthermore, degraded chemical agent may have highly undesirable or even severely toxic side effects.

Because chemical stability is a critical aspect in the design and manufacture, as well as regulatory review and approval, of pharmaceutical compositions and dosage forms, in recent years extensive and systematic studies have been conducted on the mechanisms and kinetics of decomposition of pharmaceutical agents. For a brief review, see Alfred Martin,

Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences, 4th Edition, pp. 305-312 (Lee & Febiger, Philadelphia, 1993).

B. Prior Methods for Increasing the Stability of a Chemical Compound in Pharmaceutically Acceptable Formulations

Various methods have been devised to achieve improved chemical stability of a compound, including alteration of environmental parameters, such as buffer type, pH, storage temperature, and elimination of catalytic ions or ions necessary for enzyme activity using chelating agents.

Other methods include converting the drug into a more stable prodrug which, under physiological conditions, is processed to become a biologically active form of the compound.

Another method for improving the chemical stability of pharmaceutical agents employs novel dosage form designs. Dosage form designs that improve the chemical stability of a drug include loading drugs into liposomes or polymers, *e.g.*, during emulsion polymerization. However, such techniques have problems and limitations. For example, a lipid soluble drug is often required to prepare a suitable liposome. Further, unacceptably large amounts of the liposome or polymer may be required to prepare unit drug doses. Further still, techniques for preparing such pharmaceutical compositions tend to be complex. Finally, removal of contaminants at the end of the emulsion polymerization manufacturing process, such as potentially toxic unreacted monomer or initiator, can be difficult and expensive.

Another example of a dosage form that can be used to increase the stability of an administered agent is a monolithic device, which is a rate-controlling polymer matrix throughout which a drug is dissolved or dispersed. Yet another example of such a dosage form is a reservoir device, which is a shell-like dosage form having a drug contained within a rate-controlling membrane.

An exemplary reservoir dosage form is described in U.S. Patent No. 4,725,442, which refers to water insoluble drug materials solubilized in an organic liquid and incorporated in microcapsules of phospholipids. One disadvantage of this dosage form is the toxic effects of the solubilizing organic liquids. Other methods of forming reservoir dosage forms of pharmaceutical drug microcapsules include micronizing a slightly-soluble drug by high-speed

stirring or impact comminution of a mixture of the drug and a sugar or sugar alcohol together with suitable excipients or diluents. *See e.g.* EP 411,629A. One disadvantage of this method is that the resultant drug particles are larger than those obtained with milling. Yet another method of forming a reservoir dosage form is directed to polymerization of a monomer in the presence of an active drug material and a surfactant to produce small-particle microencapsulation (*International Journal of Pharmaceutics*, **52**:101-108 (1989)). This process, however, produces compositions containing contaminants, such as toxic monomers, which are difficult to remove. Complete removal of such monomers can be expensive, particularly when conducted on a manufacturing scale. A reservoir dosage form can also be formed by co-dispersion of a drug or a pharmaceutical agent in water with droplets of a carbohydrate polymer (*see e.g.* U.S. Patent No. 4,713,249 and WO 84/00294). The major disadvantage of this procedure is that in many cases, a solubilizing organic co-solvent is required for the encapsulation procedure. Removal of traces of such harmful co-solvents can result in an expensive manufacturing process.

C. Background Regarding Nanoparticulate Compositions

Nanoparticulate compositions, first described in U.S. Patent No. 5,145,684 (“the ‘684 patent”), are particles consisting of a poorly soluble active agent having adsorbed onto the surface thereof a non-crosslinked surface stabilizer. The ‘684 patent also describes methods of making such nanoparticulate compositions. The ‘684 patent teaches that nanoparticulate compositions are desirable because with a decrease in particle size, and a consequent increase in surface area, a composition can exhibit superior bioavailability.

Methods of making nanoparticulate compositions are described, for example, in U.S. Patent Nos. 5,518,187 and 5,862,999, both for “Method of Grinding Pharmaceutical Substances;” U.S. Patent No. 5,718,388, for “Continuous Method of Grinding Pharmaceutical Substances;” and U.S. Patent No. 5,510,118 for “Process of Preparing Therapeutic Compositions Containing Nanoparticles.”

Nanoparticulate compositions are also described, for example, in U.S. Patent Nos. 5,298,262 for “Use of Ionic Cloud Point Modifiers to Prevent Particle Aggregation During Sterilization;” 5,302,401 for “Method to Reduce Particle Size Growth During

Lyophilization;" 5,318,767 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,326,552 for "Novel Formulation For Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" 5,328,404 for "Method of X-Ray Imaging Using Iodinated Aromatic Propanedioates;" 5,336,507 for "Use of Charged Phospholipids to Reduce Nanoparticle Aggregation;" 5,340,564 for "Formulations Comprising Olin 10-G to Prevent Particle Aggregation and Increase Stability;" 5,346,702 for "Use of Non-Ionic Cloud Point Modifiers to Minimize Nanoparticulate Aggregation During Sterilization;" 5,349,957 for "Preparation and Magnetic Properties of Very Small Magnetic-Dextran Particles;" 5,352,459 for "Use of Purified Surface Modifiers to Prevent Particle Aggregation During Sterilization;" 5,399,363 and 5,494,683, both for "Surface Modified Anticancer Nanoparticles;" 5,401,492 for "Water Insoluble Non-Magnetic Manganese Particles as Magnetic Resonance Enhancement Agents;" 5,429,824 for "Use of Tyloxapol as a Nanoparticulate Stabilizer;" 5,447,710 for "Method for Making Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" 5,451,393 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,466,440 for "Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents in Combination with Pharmaceutically Acceptable Clays;" 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation;" 5,472,683 for "Nanoparticulate Diagnostic Mixed Carbamic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,500,204 for "Nanoparticulate Diagnostic Dimers as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,518,738 for "Nanoparticulate NSAID Formulations;" 5,521,218 for "Nanoparticulate Iododipamide Derivatives for Use as X-Ray Contrast Agents;" 5,525,328 for "Nanoparticulate Diagnostic Diatrizoxy Ester X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" 5,552,160 for "Surface Modified NSAID Nanoparticles;" 5,560,931 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,565,188 for "Polyalkylene Block Copolymers as Surface Modifiers for Nanoparticles;" 5,569,448 for "Sulfated Non-ionic Block Copolymer Surfactant as Stabilizer Coatings for Nanoparticle Compositions;" 5,571,536 for "Formulations of

Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,573,749 for "Nanoparticulate Diagnostic Mixed Carboxylic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,573,750 for "Diagnostic Imaging X-Ray Contrast Agents;" 5,573,783 for "Redispersible Nanoparticulate Film Matrices With Protective Overcoats;" 5,580,579 for "Site-specific Adhesion Within the GI Tract Using Nanoparticles Stabilized by High Molecular Weight, Linear Poly(ethylene Oxide) Polymers;" 5,585,108 for "Formulations of Oral Gastrointestinal Therapeutic Agents in Combination with Pharmaceutically Acceptable Clays;" 5,587,143 for "Butylene Oxide-Ethylene Oxide Block Copolymers Surfactants as Stabilizer Coatings for Nanoparticulate Compositions;" 5,591,456 for "Milled Naproxen with Hydroxypropyl Cellulose as Dispersion Stabilizer;" 5,593,657 for "Novel Barium Salt Formulations Stabilized by Non-ionic and Anionic Stabilizers;" 5,622,938 for "Sugar Based Surfactant for Nanocrystals;" 5,628,981 for "Improved Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents and Oral Gastrointestinal Therapeutic Agents;" 5,643,552 for "Nanoparticulate Diagnostic Mixed Carbonic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" 5,718,919 for "Nanoparticles Containing the R(-)Enantiomer of Ibuprofen;" 5,747,001 for "Aerosols Containing Beclomethasone Nanoparticle Dispersions;" 5,834,025 for "Reduction of Intravenously Administered Nanoparticulate Formulation Induced Adverse Physiological Reactions;" 6,045,829 "Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,068,858 for "Methods of Making Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,153,225 for "Injectable Formulations of Nanoparticulate Naproxen;" 6,165,506 for "New Solid Dose Form of Nanoparticulate Naproxen;" 6,221,400 for "Methods of Treating Mammals Using Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors;" 6,264,922 for "Nebulized Aerosols Containing Nanoparticle Dispersions;" 6,267,989 for "Methods for Preventing Crystal Growth and Particle Aggregation in Nanoparticle Compositions;" 6,270,806 for "Use of PEG-Derivatized Lipids as Surface Stabilizers for Nanoparticulate Compositions;" 6,316,029 for "Rapidly Disintegrating Solid Oral Dosage Form," 6,375,986

for “Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate;” 6,428,814 for “Bioadhesive nanoparticulate compositions having cationic surface stabilizers;” and 6,432,381 for “Methods for targeting drug delivery to the upper and/or lower gastrointestinal tract,” all of which are specifically incorporated by reference. In addition, U.S. Patent Application No. 20020012675 A1, published on January 31, 2002, for “Controlled Release Nanoparticulate Compositions,” describes nanoparticulate compositions, and is specifically incorporated by reference.

Amorphous small particle compositions are described, for example, in U.S. Patent Nos. 4,783,484 for “Particulate Composition and Use Thereof as Antimicrobial Agent;” 4,826,689 for “Method for Making Uniformly Sized Particles from Water-Insoluble Organic Compounds;” 4,997,454 for “Method for Making Uniformly-Sized Particles From Insoluble Compounds;” 5,741,522 for “Ultrasmall, Non-aggregated Porous Particles of Uniform Size for Entrapping Gas Bubbles Within and Methods;” and 5,776,496, for “Ultrasmall Porous Particles for Enhancing Ultrasound Back Scatter.”

In particular, U.S. Patent Nos. 5,399,363 and 5,494,683, both for “Surface Modified Anticancer Nanoparticles,” refer to nanoparticulate compositions of anticancer agents, including taxol, also known as paclitaxel. However, these patents do not address a means to overcome the chemical instability of an active agent, such as paclitaxel at a basic pH. Specifically, the two patents teach methods of making nanoparticulate paclitaxel compositions which are stable at a pH of 7.4. However, this does not teach or suggest that a nanoparticulate paclitaxel composition is stable at an *elevated* pH, such as a basic pH of 9.

These references describe methods of increasing the bioavailability of poorly soluble active agents, in which the class of “active agents” encompassed is limited only by the solubility of the active agent. In contrast, the present invention is directed to a class of poorly soluble active agents which are unstable under one or more environmental conditions. The two classes of active agents are not the same, as all poorly soluble active agents are not unstable under one or more environmental conditions.

This is significant as the pH varies along the gastrointestinal tract (GIT). If a cancer is localized in a region of the GIT which has a basic pH, the conventional form of paclitaxel

will be ineffective, or poorly effective, as the drug degrades at a high pH. This problem can be addressed by nanoparticulate forms of paclitaxel, which are stable at a high pH.

U.S. Patent No. 5,989,591 to Nagi, for "Rapamycin Formulations for Oral Administration," refers to solid dose compositions of rapamycin in which the rapamycin can have a nanoparticulate particle size. *See* Nagi at col. 6, lines 50-55. However, Nagi does not teach that the nanoparticulate rapamycin composition is stable when exposed to an aqueous environment. In fact, Nagi teaches that the nanoparticulate rapamycin composition is formulated into a tablet dosage form, in which rapamycin is present in a sugar coating over a solid core. *See* the Abstract and col. 3, lines 45-52.

This does not teach or suggest that an unstable form of rapamycin, *i.e.*, rapamycin exposed to an aqueous environment, could be selected and made stable under that same environment by formulating the drug into a nanoparticulate composition. This is significant as tablet dosage forms have a longer onset of activity as compared to liquid or IV dosage forms. In addition, many infant and elderly patients are unable to swallow tablet dosage forms.

* * * *

There is a need in the art for a method of stabilizing active agents, which is efficient, cost-effective, and does not require the addition of potentially toxic solvents. The present invention satisfies this need.

SUMMARY OF THE INVENTION

The present invention is directed to the discovery that active agents which are unstable under one or more environmental conditions, when formulated into nanoparticulate compositions, are stable under those same environmental conditions. Such environmental conditions can be, for example, exposure to a condition such as prolonged storage periods, elevated temperature, non-physiological pH, light, radiation, radiation causing photolysis, enzymes or other catalysts, water, solvent molecules, oxidizing agents, free radicals, and freezing-thawing temperature cycles.

One aspect of the invention is directed to a process for stabilizing active agents, particularly pharmaceutical agents, comprising formulating an active agent which is unstable under one or more environmental conditions into a nanoparticulate composition, wherein the active agent is then stable under those same environmental conditions. The nanoparticulate composition comprises a poorly soluble active agent, such as a drug particle, and one or more non-crosslinked surface stabilizers adsorbed on to the surface of the active agent. The nanoparticulate compositions have an effective average particle size of less than about two microns.

The present invention is further directed to a process for stabilizing rapamycin, which is unstable when exposed to water, comprising forming a nanoparticulate formulation of rapamycin having one or more non-crosslinked surface stabilizers adsorbed on to the surface of the drug. The resultant nanoparticulate rapamycin composition exhibits dramatically superior stability when exposed to water. The pharmaceutical composition preferably comprises a pharmaceutically acceptable carrier, as well as any desired excipients.

Yet another aspect of the invention encompasses a process for stabilizing paclitaxel, which is unstable when exposed to a basic pH, comprising forming a nanoparticulate composition of paclitaxel having one or more non-crosslinked surface stabilizers adsorbed on to the surface of the drug. The resultant nanoparticulate paclitaxel composition exhibits dramatically superior stability when exposed to a basic pH. The pharmaceutical composition preferably comprises a pharmaceutically acceptable carrier, as well as any desired excipients.

Both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1: Shows the effect of 0.005 N NaOH (a basic pH level) on the rate of degradation of paclitaxel as compared to the rate of degradation of a nanoparticulate formulation of paclitaxel.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a method for stabilizing active agents, particularly pharmaceutical agents, which are unstable under one or more environmental conditions, comprising formulating an active agent into a nanoparticulate composition. The nanoparticulate active agent is stable under the same one or more environmental conditions under which the active agent is unstable prior to formulation into a nanoparticulate composition. Such one or more environmental conditions which can cause the active agent to degrade include, but are not limited to, prolonged storage periods, elevated temperature, non-physiological pH, light, radiation, radiation causing photolysis, enzymes or other catalysts, water, solvent molecules, oxidizing agents, free radicals, and freezing-thawing temperature cycles.

The method of the invention comprises first identifying and selecting an active agent which is unstable under one or more environmental conditions, followed by formulating particles of the active agent into a stable nanoparticulate composition. The formulation step comprises combining the active agent particles and at least one non-crosslinked surface stabilizer, wherein after combining the at least one non-crosslinked surface stabilizer adsorbs to the surface of the active agent particles to produce a nanoparticulate composition in which the active agent particles have an effective average particle size of less than about 2 microns.

The claimed method is distinct from prior art methods of making nanoparticulate compositions, as prior methods did not teach that active agents which are unstable under one or more environmental conditions can be made stable under *those same conditions* by formulating the active agent into a nanoparticulate composition. Rather, prior methods of making nanoparticulate compositions teach that the nanoparticulate compositions are desirable as they can increase the bioavailability of the component active agent. *See e.g.*, U.S. Patent No. 5,145,684.

This distinction is significant as the present invention can expand the use of many active agents which may have only been useable under limited conditions prior to the present invention. For example, active agents which are stable only under certain pH conditions, such as paclitaxel, can be formulated for administration at any desired pH level. This is desirable as the pH varies along the human gastrointestinal tract. Similarly, active agents

which are unstable when exposed to elevated temperatures can be heat sterilized without degradation when formulated into a nanoparticulate composition. This can be useful in administering active agents to very young or immunocompromised patients. Moreover, active agents which are unstable when exposed to water (*i.e.*, hydrolysis), such as rapamycin, can be formulated for administration in a liquid form. This can be beneficial as tablet dosage forms have a longer onset of activity as compared to liquid or IV dosage forms. In addition, many infant and elderly patients are unable to swallow tablet dosage forms.

**A. Active Agents Formulated into Nanoparticulate Compositions
Exhibit Increased Stability of the Component Active Agent**

Chemical instability due to degradation is usually a result of hydrolysis, oxidation, isomerization, epimerization, or photolysis. Apart from the structural, physical, and chemical nature of the active agent, the rate of degradation is often determined by numerous environmental factors, including temperature, light, radiation, enzyme or other catalysts, pH and ionic strength of the solution, solvent type, or buffer species.

While not intending to be bound by theory, one possibility is that the molecules of the surface stabilizer shield the active agent, thereby protecting potentially labile chemical groups of the active agent from the potentially hostile environment. Another possibility is that for a crystalline active agent particle, the crystalline structure in a nanoparticulate sized formulation results in greater active agent stability.

For example, rapamycin is rapidly degraded when exposed to an aqueous environment. The main degradation scheme of rapamycin is the cleavage of the macrocyclic lactone ring by the hydrolysis of an ester bond to form a secoacid (SECO). The secoacid undergoes further dehydration and isomerization to form diketomorpholine analogs.

However, as described in the examples below, when rapamycin is formulated into a nanoparticulate composition, minimal or no rapamycin degradation is observed, even following prolonged exposure to an aqueous medium.

Another example of an active agent that is unstable under certain environmental conditions, but which is stable in a nanoparticulate formulation under those same environmental conditions, is paclitaxel. Upon exposure to a basic pH (*i.e.*, a pH of about 9), paclitaxel rapidly degrades. Ringel et al., *J. Pharmac. Exp. Ther.*, 242:692-698 (1987).

However, when paclitaxel is formulated into a nanoparticulate composition, minimal or no paclitaxel degradation is observed, even when the composition is exposed to a basic pH.

B. Methods of Preparing Nanoparticulate Compositions

1. Active Agent and Surface Stabilizer Components

The method of stabilizing an active agent according to the present invention comprises formulating the active agent into a nanoparticulate formulation. The nanoparticulate active agent formulation comprises at least one active agent and one or more surface stabilizers adsorbed to the surface of the active agent(s).

a. Active Agent Particles

The nanoparticles of the invention comprise an active agent, such as a therapeutic or diagnostic agent, having one or more labile groups or exhibiting chemical instability when exposed to certain environmental conditions, such as prolonged storage periods, elevated temperature, non-physiological pH, light, radiation, radiation causing photolysis, enzymes or other catalysts, water (which can cause hydrolysis), solvent molecules, oxidizing agents, free radicals, and freezing-thawing temperature cycles. In addition, active agents having, for example, cosmetic, diagnostic, or bioengineering uses are presumed suitable for the invention.

The active agent may be present either substantially in the form of one optically pure enantiomer or as a mixture, racemic or otherwise, of enantiomers.

A therapeutic agent can be a pharmaceutical, including biologics such as proteins and peptides, and a diagnostic agent is typically a contrast agent, such as an x-ray contrast agent, or any other type of diagnostic material. The active agent particle exists as a discrete, crystalline phase, amorphous phase, semi-crystalline phase, semi-amorphous phase, or as a combination thereof. The crystalline phase differs from a non-crystalline or amorphous phase which results from precipitation techniques, such as those described in EP Patent No. 275,796.

The active agent is preferably present in an essentially pure form, is poorly soluble, and is dispersible in at least one liquid medium. By "poorly soluble" it is meant that the active agent has a solubility in a liquid dispersion medium of less than about 30 mg/mL, less

than about 10 mg/mL, or less than about 1 mg/mL. The dispersion medium can be, for example, water, safflower oil, ethanol, t-butanol, glycerin, polyethylene glycol (PEG), hexane, or glycol.

The active agent can be selected from a variety of known classes of drugs, including, for example, COX-2 inhibitors, retinoids, NSAIDS, proteins, peptides, nucleotides, anti-obesity drugs, nutraceuticals, dietary supplements, carotenoids, corticosteroids, elastase inhibitors, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, alpha-hydroxy formulations, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, and respiratory illness therapies associated with acquired immune deficiency syndrome.

Exemplary nutraceuticals and dietary supplements are disclosed, for example, in Roberts et al., *Nutraceuticals: The Complete Encyclopedia of Supplements, Herbs, Vitamins, and Healing Foods* (American Nutraceutical Association, 2001), which is specifically incorporated by reference. A nutraceutical or dietary supplement, also known as phytochemicals or functional foods, is generally any one of a class of dietary supplements, vitamins, minerals, herbs, or healing foods that have medical or pharmaceutical effects on the body. Exemplary nutraceuticals or dietary supplements include, but are not limited to, lutein,

folic acid, fatty acids (*e.g.*, DHA and ARA), fruit and vegetable extracts, vitamin and mineral supplements, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids (*e.g.*, iso-leucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish and marine animal oils, and probiotics. Nutraceuticals and dietary supplements also include bio-engineered foods genetically engineered to have a desired property, also known as “pharmafoods.”

A description of these classes of active agents and a listing of species within each class can be found in Martindale, *The Extra Pharmacopoeia*, Twenty-ninth Edition (The Pharmaceutical Press, London, 1989), specifically incorporated by reference. The active agents are commercially available and/or can be prepared by techniques known in the art.

b. Surface Stabilizers

Exemplary useful surface stabilizers include, but are not limited to, known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products, and surfactants. Preferred surface stabilizers include nonionic, cationic, and ionic surfactants. Combinations of more than one surface stabilizer can be used in the invention. The surface stabilizers do not chemically interact with the active agent particles, and individually adsorbed molecules of the surface stabilizer are essentially free of intermolecular crosslinkages.

Representative examples of surface stabilizers include hydroxypropyl methylcellulose, hydroxypropylcellulose, polyvinylpyrrolidone, random copolymers of vinyl pyrrolidone and vinyl acetate, sodium lauryl sulfate, dioctylsulfosuccinate, gelatin, casein, lecithin (phosphatides), dextran, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers (*e.g.*, macrogol ethers such as cetomacrogol 1000), polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters (*e.g.*, the commercially available Tweens[®] such as *e.g.*, Tween 20[®] and Tween 80[®] (ICI Speciality Chemicals)); polyethylene glycols (*e.g.*, Carbowax 3550[®] and

934[®] (Union Carbide)), polyoxyethylene stearates, colloidal silicon dioxide, phosphates, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminium silicate, triethanolamine, polyvinyl alcohol (PVA), 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol, superione, and triton), poloxamers (*e.g.*, Pluronic F68[®] and F108[®], which are block copolymers of ethylene oxide and propylene oxide); poloxamines (*e.g.*, Tetronic 908[®], also known as Poloxamine 908[®], which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Wyandotte Corporation, Parsippany, N.J.)); Tetronic 1508[®] (T-1508) (BASF Wyandotte Corporation), Tritons X-200[®], which is an alkyl aryl polyether sulfonate (Rohm and Haas); Crodestas F-110[®], which is a mixture of sucrose stearate and sucrose distearate (Croda Inc.); p-isononylphenoxypoly-(glycidol), also known as Olin-10G[®] or Surfactant 10-G[®] (Olin Chemicals, Stamford, CT); Crodestas SL-40[®] (Croda, Inc.); and SA9OHCO, which is C₁₈H₃₇CH₂C(O)N(CH₃)-CH₂(CHOH)₄(CH₂OH)₂ (Eastman Kodak Co.); decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-nonyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; PEG-phospholipid, PEG-cholesterol, PEG-cholesterol derivative, PEG-vitamin A, PEG-vitamin E, lysozyme, and the like.

Examples of useful cationic surface stabilizers include, but are not limited to, polymers, biopolymers, polysaccharides, cellulose, alginates, phospholipids, and nonpolymeric compounds, such as zwitterionic stabilizers, poly-n-methylpyridinium, anthryl pyridinium chloride, cationic phospholipids, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide (PMMTMABr), hexyldesyltrimethylammonium bromide (HDMAB), and polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate.

Other useful cationic stabilizers include, but are not limited to, cationic lipids, sulfonium, phosphonium, and quaternary ammonium compounds, such as

stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride or bromide, coconut methyl dihydroxyethyl ammonium chloride or bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride or bromide, C₁₂₋₁₅dimethyl hydroxyethyl ammonium chloride or bromide, coconut dimethyl hydroxyethyl ammonium chloride or bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride or bromide, lauryl dimethyl (ethenoxy)₄ ammonium chloride or bromide, N-alkyl (C₁₂₋₁₈)dimethylbenzyl ammonium chloride, N-alkyl (C₁₄₋₁₈)dimethyl-benzyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C₁₂₋₁₄) dimethyl 1-naphthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts and dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt and/or an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C₁₂₋₁₄) dimethyl 1-naphthylmethyl ammonium chloride and dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C₁₂, C₁₅, C₁₇ trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyl dimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride (ALQUAT 336™), POLYQUAT 10™, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters (such as choline esters of fatty acids), benzalkonium chloride, stearylalkonium chloride compounds (such as stearyltrimonium chloride and Di-stearyldimonium chloride), cetyl pyridinium bromide or chloride, halide salts of quaternized polyoxyethylalkylamines, MIRAPOL™ and ALKAQUAT™ (Alkaril Chemical Company), alkyl pyridinium salts; amines, such as alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, and vinyl pyridine, amine salts, such as lauryl amine acetate, stearyl amine acetate, alkylpyridinium salt, and

alkylimidazolium salt, and amine oxides; imide azolinium salts; protonated quaternary acrylamides; methylated quaternary polymers, such as poly[diallyl dimethylammonium chloride] and poly-[N-methyl vinyl pyridinium chloride]; and cationic guar.

Such exemplary cationic surface stabilizers and other useful cationic surface stabilizers are described in J. Cross and E. Singer, *Cationic Surfactants: Analytical and Biological Evaluation* (Marcel Dekker, 1994); P. and D. Rubingh (Editor), *Cationic Surfactants: Physical Chemistry* (Marcel Dekker, 1991); and J. Richmond, *Cationic Surfactants: Organic Chemistry*, (Marcel Dekker, 1990).

Particularly preferred nonpolymeric primary stabilizers are any nonpolymeric compound, such benzalkonium chloride, a carbonium compound, a phosphonium compound, an oxonium compound, a halonium compound, a cationic organometallic compound, a quaternary phosphorous compound, a pyridinium compound, an anilinium compound, an ammonium compound, a hydroxylammonium compound, a primary ammonium compound, a secondary ammonium compound, a tertiary ammonium compound, and quaternary ammonium compounds of the formula $\text{NR}_1\text{R}_2\text{R}_3\text{R}_4^{(+)}$. For compounds of the formula $\text{NR}_1\text{R}_2\text{R}_3\text{R}_4^{(+)}$:

- (i) none of $\text{R}_1\text{-R}_4$ are CH_3 ;
- (ii) one of $\text{R}_1\text{-R}_4$ is CH_3 ;
- (iii) three of $\text{R}_1\text{-R}_4$ are CH_3 ;
- (iv) all of $\text{R}_1\text{-R}_4$ are CH_3 ;
- (v) two of $\text{R}_1\text{-R}_4$ are CH_3 , one of $\text{R}_1\text{-R}_4$ is $\text{C}_6\text{H}_5\text{CH}_2$, and one of $\text{R}_1\text{-R}_4$ is an alkyl chain of seven carbon atoms or less;
- (vi) two of $\text{R}_1\text{-R}_4$ are CH_3 , one of $\text{R}_1\text{-R}_4$ is $\text{C}_6\text{H}_5\text{CH}_2$, and one of $\text{R}_1\text{-R}_4$ is an alkyl chain of nineteen carbon atoms or more;
- (vii) two of $\text{R}_1\text{-R}_4$ are CH_3 and one of $\text{R}_1\text{-R}_4$ is the group $\text{C}_6\text{H}_5(\text{CH}_2)_n$, where $n > 1$;
- (viii) two of $\text{R}_1\text{-R}_4$ are CH_3 , one of $\text{R}_1\text{-R}_4$ is $\text{C}_6\text{H}_5\text{CH}_2$, and one of $\text{R}_1\text{-R}_4$ comprises at least one heteroatom;
- (ix) two of $\text{R}_1\text{-R}_4$ are CH_3 , one of $\text{R}_1\text{-R}_4$ is $\text{C}_6\text{H}_5\text{CH}_2$, and one of $\text{R}_1\text{-R}_4$ comprises at least one halogen;

- (x) two of R_1 - R_4 are CH_3 , one of R_1 - R_4 is $C_6H_5CH_2$, and one of R_1 - R_4 comprises at least one cyclic fragment;
- (xi) two of R_1 - R_4 are CH_3 and one of R_1 - R_4 is a phenyl ring; or
- (xii) two of R_1 - R_4 are CH_3 and two of R_1 - R_4 are purely aliphatic fragments.

Such compounds include, but are not limited to, behenalkonium chloride, benzethonium chloride, cetylpyridinium chloride, behentrimonium chloride, lauralkonium chloride, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cethylamine hydrofluoride, chlorallylmethenamine chloride (Quaternium-15), distearyldimonium chloride (Quaternium-5), dodecyl dimethyl ethylbenzyl ammonium chloride (Quaternium-14), Quaternium-22, Quaternium-26, Quaternium-18 hectorite, dimethylaminoethylchloride hydrochloride, cysteine hydrochloride, diethanolammonium POE (10) oleyl ether phosphate, diethanolammonium POE (3) oleyl ether phosphate, tallow alkonium chloride, dimethyl dioctadecylammoniumbentonite, stearylalkonium chloride, domiphen bromide, denatonium benzoate, myristalkonium chloride, laurtrimonium chloride, ethylenediamine dihydrochloride, guanidine hydrochloride, pyridoxine HCl, iofetamine hydrochloride, meglumine hydrochloride, methylbenzethonium chloride, myrtrimonium bromide, oleyltrimonium chloride, polyquaternium-1, procaine hydrochloride, cocobetaine, stearylalkonium bentonite, stearylalkoniumhectonite, stearyl trihydroxyethyl propylenediamine dihydrofluoride, tallowtrimonium chloride, and hexadecyltrimethyl ammonium bromide.

Most of these surface stabilizers are known pharmaceutical excipients and are described in detail in the *Handbook of Pharmaceutical Excipients*, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain (The Pharmaceutical Press, 2000), specifically incorporated by reference. The surface stabilizers are commercially available and/or can be prepared by techniques known in the art.

c. Nanoparticulate Active Agent/Surface Stabilizer Particle Size

The compositions of the invention contain nanoparticulate active agent particles which have an effective average particle size of less than about 2 microns, less than about 1 micron, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than

about 300 nm, less than about 200 nm, less than about 100 nm, or less than about 50 nm, as measured by light-scattering methods, microscopy, or other appropriate methods.

By “an effective average particle size of “less than about 2 microns,” it is meant that at least 50% of the active agent particles have a weight average particle size of less than about 2 microns when measured by light scattering techniques, microscopy, or other appropriate methods. Preferably, at least 70% of the active agent particles have an average particle size of less than about 2 microns, more preferably at least 90% of the active agent particles have an average particle size of less than about 2 microns, and even more preferably at least about 95% of the particles have a weight average particle size of less than about 2 microns.

**d. Concentration of Nanoparticulate
Active Agent and Surface Stabilizer**

The relative amount of active agent and one or more surface stabilizers can vary widely. The optimal amount of the one or more surface stabilizers can depend, for example, upon the particular active agent selected, the hydrophilic lipophilic balance (HLB), melting point, and water solubility of the surface stabilizer, and the surface tension of water solutions of the surface stabilizer, *etc.*

The concentration of the at least one active agent can vary from about 99.5% to about 0.001%, from about 95% to about 0.1%, or from about 90% to about 0.5%, by weight, based on the total combined weight of the at least one active agent and at least one surface stabilizer, not including other excipients.

The concentration of the at least one surface stabilizer can vary from about 0.001 to about 99.5%, from about 0.1% to about 95%, and from about 0.5% to about 90%, by weight, based on the total combined weight of the at least one active agent and at least one surface stabilizer, not including other excipients.

2. Methods of Making Nanoparticulate Formulations

Nanoparticulate active agent compositions can be made using methods known in the art such as, for example, milling, homogenization, and precipitation techniques. Exemplary methods of making nanoparticulate compositions are described in U.S. Patent No. 5,145,684. Methods of making nanoparticulate compositions are also described in U.S. Patent Nos.

5,518,187; 5,718,388; 5,862,999; 5,665,331; 5,662,883; 5,560,932; 5,543,133; 5,534,270; 5,510,118; and 5,470,583, all referenced in the background of the invention.

1. Milling to Obtain Nanoparticulate Active Agent Dispersions

Milling an active agent to obtain a nanoparticulate composition comprises dispersing active agent particles in a liquid dispersion medium in which the active agent is poorly soluble, followed by applying mechanical means in the presence of grinding media to reduce the particle size of the active agent particles to the desired effective average particle size. The dispersion medium can be, for example, water, safflower oil, ethanol, t-butanol, glycerin, polyethylene glycol (PEG), hexane, or glycol.

The active agent particles can be reduced in size in the presence of at least one surface stabilizer. Alternatively, the active agent particles can be contacted with one or more surface stabilizers after attrition. Other compounds, such as a diluent, can be added to the active agent/surface stabilizer composition during the size reduction process. Dispersions can be manufactured continuously or in a batch mode. The resultant nanoparticulate active agent dispersion can be utilized in solid or liquid dosage formulations, such as controlled release formulations, solid dose fast melt formulations, aerosol formulations, lyophilized formulations, tablets, capsules, solid lozenge, powders, etc.

2. Precipitation to Obtain Nanoparticulate Active Agent Compositions

Another method of forming the desired nanoparticulate active agent composition is by microprecipitation. This is a method of preparing stable dispersions of poorly soluble active agents in the presence of one or more surface stabilizers and one or more colloid stability enhancing surface active agents free of any trace toxic solvents or solubilized heavy metal impurities. Such a method comprises, for example: (1) dissolving an active agent in a suitable solvent; (2) adding the formulation from step (1) to a solution comprising at least one surface stabilizer; and (3) precipitating the formulation from step (2) using an appropriate non-solvent. The method can be followed by removal of any formed salt, if present, by dialysis or diafiltration and concentration of the dispersion by conventional means. The resultant nanoparticulate active agent dispersion can be utilized in solid or liquid dosage

formulations, such as controlled release formulations, solid dose fast melt formulations, aerosol formulations, lyophilized formulations, tablets, solid lozenge, powders, capsules, etc.

3. Homogenization to Obtain Active Agent Nanoparticulate Compositions

Exemplary homogenization methods of preparing active agent nanoparticulate compositions are described in U.S. Patent No. 5,510,118, for "Process of Preparing Therapeutic Compositions Containing Nanoparticles."

C. Methods of Using Nanoparticulate Active Agent Formulations

The nanoparticulate compositions of the present invention can be administered to humans and animals either orally, rectally, parenterally (intravenous, intramuscular, or subcutaneous), intracisternally, intravaginally, intraperitoneally, locally (powders, ointments or drops), or as a buccal or nasal spray.

Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate.

Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. The nanoparticulate compositions may also contain adjuvants, such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the growth of microorganisms can be ensured by various antibacterial and antifungal agents, such as parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, such as aluminum monostearate and gelatin.

Solid dosage forms for oral administration include capsules, tablets, pills,

powders, and granules. In such solid dosage forms, the active compound is admixed with at least one of the following: (a) one or more inert excipients (or carrier), such as sodium citrate or dicalcium phosphate; (b) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (c) binders, such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and acacia; (d) humectants, such as glycerol; (e) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate; (f) solution retarders, such as paraffin; (g) absorption accelerators, such as quaternary ammonium compounds; (h) wetting agents, such as cetyl alcohol and glycerol monostearate; (i) adsorbents, such as kaolin and bentonite; and (j) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. For capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage forms may comprise inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers. Exemplary emulsifiers are ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, such as cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, fatty acid esters of sorbitan, or mixtures of these substances, and the like.

Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Actual dosage levels of active agent in the nanoparticulate compositions of the invention may be varied to obtain an amount of active agent that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired therapeutic effect, on the route of administration, on the desired duration of treatment, and other factors.

The total daily dose of the compounds of this invention administered to a host in single or divided dose may be in amounts of, for example, from about 1 nanomole to about 5 micromoles per kilogram of body weight. Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the body weight, general health, sex, diet, time and route of administration, rates of absorption and excretion, combination with other active agents, and the severity of the particular disease being treated.

The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to publicly available documents are specifically incorporated by reference.

Example 1

The purpose of this example was to determine the effect on the stability of paclitaxel at a basic pH when the drug is formulated into a nanoparticulate composition.

Paclitaxel is a naturally occurring diterpenoid which has demonstrated great potential as an anti-cancer drug. Paclitaxel can be isolated from the bark of the western yew, *Taxus brevifolia*, and is also found in several other yew species such as *T. baccata* and *T. cuspidata*. Upon exposure to a basic pH (*i.e.*, a pH of about 9), the drug rapidly degrades. Ringel et al., *J. Pharmac. Exp. Ther.*, 242:692-698 (1987).

Two formulations of paclitaxel were prepared: a solubilized formulation of paclitaxel and a nanoparticulate formulation of paclitaxel. The degradation of paclitaxel for both formulations was then compared. For Formulation I, paclitaxel (Biolyse; Quebec, Canada) was solubilized in 1% methanol and 99% H₂O to make a 2% paclitaxel solution. Formulation II was prepared by milling the 2% paclitaxel solution with 1% Plurionic F108™ (BASF) in a 0.5 oz amber bottle containing 7.5 ml 0.5 mm Yttria-doped Zirconia media on a U.S. Stoneware Roller Mill for 72 hours. The resultant milled paclitaxel composition had an effective average particle size of about 220 nm, as measured by a Coulter Counter (Coulter Electronics Inc.).

Both solubilized paclitaxel (Formulation I) and nanoparticulate paclitaxel (Formulation II) were incubated with 0.005 N NaOH solution (a basic solution). At the end of the incubation period, base degradation of paclitaxel was stopped by adding to the incubation solution 1/100 its volume of 1N HCl. The recovery of paclitaxel was then measured at various time periods by HPLC.

As shown in Figure 1, solubilized paclitaxel rapidly degraded when exposed to basic pH conditions, as only about 20% of the paclitaxel was recoverable after a 20 minute incubation period. In contrast, nanoparticulate paclitaxel was essentially stable under basic conditions, as more than 90% of the drug was recoverable after the same incubation period.

Example 2

The purpose of this example was to determine the stability of rapamycin when the drug is formulated into a nanoparticulate composition.

Rapamycin is useful as an immunosuppressant and as an antifungal antibiotic, and its use is described in, for example, U.S. Patent Nos. 3,929,992, 3,993,749, and 4,316,885, and in Belgian Pat. No. 877,700. The compound, which is only slightly soluble in water, *i.e.*, 20 micrograms per mL, rapidly hydrolyzes when exposed to water. Because rapamycin is highly unstable when exposed to an aqueous medium, special injectable formulations have been developed for administration to patients, such as those described in European Patent No. EP 041,795. Such formulations are often undesirable, as frequently the non-aqueous solubilizing agent exhibits toxic side effects.

Two different formulations of rapamycin were prepared and then exposed to different environmental conditions. The degradation of rapamycin for each of the formulations was then compared. The two formulations were prepared as follows:

- (1) Formulation I, a mixture of 5% rapamycin and 2.5% Plurionic F68™ (BASF) in an aqueous medium; and
- (2) Formulation II, a mixture of 5% rapamycin and 1.25% Plurionic F108™ (BASF) in an aqueous medium.

Each of the two formulations was milled for 72 hours in a 0.5 ounce bottle containing 0.4 mm Yttria beads (Performance Ceramics Media) on a U.S. Stoneware Mill. Particle sizes of the resultant nanoparticulate compositions were measured by a Coulter Counter (Model No. N4MD). Following milling, Formulations I and II had effective average particle sizes of 162 nm and 171 nm, respectively.

The samples were then diluted to about 2% rapamycin with Water For Injection (WFI), bottled, and then either stored at room temperature or frozen upon completion of milling and then thawed and stored at room temperature. After ten days of storage at room temperature, Formulations I and II had effective average particle sizes of 194 nm and 199 nm, respectively.

The strength of the rapamycin in the formulations was measured by HPLC, the results of which are shown below in Table I.

TABLE I**Stability of Nanoparticulate Rapamycin under Different Storage Conditions**

Sample	Description	Storage Conditions	Storage Time	Ending Strength/ Starting Strength	SECO %*
1	Formulation I	RT	2 days	97%	<detection limit
2	Formulation II	RT	2 days	99%	<detection limit
3	Formulation III	RT	2 days	96%	<detection limit
7	Formulation I	Frozen/thawed	2 days	95%	<detection limit
8	Formulation II	Frozen/thawed	2 days	98%	<detection limit
9	Formulation III	Frozen/thawed	2 days	97%	<detection limit
1	Formulation I	RT	3 wks	95%	<detection limit
2	Formulation II	RT	3 wks	98%	<detection limit
3	Formulation III	RT	3 wks	98%	<detection limit

*SECO, or secoacid, is the primary degradation product of rapamycin. The detection limit is 0.2%.

The results show that the nanoparticulate rapamycin formulation exhibited minimal degradation of rapamycin following prolonged storage periods or exposure to the environmental conditions of freezing and thawing.

Example 3

The purpose of this example was to determine the effect of rapamycin concentration on the chemical stability of rapamycin in a nanoparticulate formulation following autoclaving.

Three rapamycin formulations were prepared by milling the following three slurries in a 250 ml Pyrex™ bottle containing 125 ml 0.4 mm Ytria-doped Zirconia media for 72 hours on a U.S. Stoneware roller mill:

- (a) 5% rapamycin/1.25% Plurionic F68™
- (b) 5% rapamycin/2.5% Plurionic F68™
- (c) 5% rapamycin/5% Plurionic F68™

Each of the three dispersions was then diluted with water to prepare formulations having rapamycin concentrations of 4.4%, 2.2%, 1.1% and 0.5% as follows:

- (1) Formulation 1: a mixture of 4.4% rapamycin and, prior to dilution, 1.25% Plurionic F68™ in an aqueous medium;

- (2) Formulation 2: a mixture of 4.4% rapamycin and, prior to dilution, 2.5% Plurionic F68™ in an aqueous medium;
- (3) Formulation 3: a mixture of 4.4% rapamycin and, prior to dilution, 5% Plurionic F68™ in an aqueous medium;
- (4) Formulation 4: a mixture of 2.2% rapamycin and, prior to dilution, 1.25% Plurionic F68™ in an aqueous medium;
- (5) Formulation 5: a mixture of 2.2% rapamycin and, prior to dilution, 2.5% Plurionic F68™ in an aqueous medium;
- (6) Formulation 6: a mixture of 2.2% rapamycin and, prior to dilution, 5% Plurionic F68™ in an aqueous medium;
- (7) Formulation 7: a mixture of 1.1% rapamycin and, prior to dilution, 1.25% Plurionic F68™ in an aqueous medium;
- (8) Formulation 8: a mixture of 1.1% rapamycin and, prior to dilution, 2.5% Plurionic F68™ in an aqueous medium;
- (9) Formulation 9: a mixture of 1.1% rapamycin and, prior to dilution, 5% Plurionic F68™ in an aqueous medium;
- (10) Formulation 10: a mixture of 0.55% rapamycin and, prior to dilution, 1.25% Plurionic F68™ in an aqueous medium;
- (11) Formulation 11: a mixture of 0.55% rapamycin and, prior to dilution, 2.5% Plurionic F68™ in an aqueous medium; and
- (12) Formulation 12: a mixture of 0.55% rapamycin and, prior to dilution, 5% Plurionic F68™ in an aqueous medium;

All twelve of the nanoparticulate formulations were autoclaved for 25 minutes at 121°C. The formulations were then stored at 4°C for 61 days, followed by testing for rapamycin degradation. No degradation, as measured by the percent of the SECO degradation product, was detected for any of the formulations.

Example 4

The purpose of this example was to determine the chemical stability of a nanoparticulate rapamycin formulation following a prolonged storage period at room temperature.

A mixture of 20% rapamycin and 10% Plurionic F68™ in an aqueous medium was milled with 0.4 mm YTZ media (Performance Ceramic Co.) on a U.S. Stoneware mill for 72 hours at room temperature. The final nanoparticulate composition had a mean particle size of between 180 to 230 nm, as measured by Coulter sizing.

After two weeks of storage at room temperature, no SECO degradation product was detected in any of the nanoparticulate preparations, indicating that there was minimal or no degradation of rapamycin in the stored nanoparticulate formulation samples.

Example 5

The purpose of this example was to determine the effect of long term storage on the chemical stability of rapamycin in a nanoparticulate composition.

Three different nanoparticulate rapamycin formulations were prepared as follows: Formulation 1, having a rapamycin concentration of 182.8 mg/mL; Formulation 2, having a rapamycin concentration of 191.4 mg/mL; and Formulation 3, having a rapamycin concentration of 192.7 mg/mL.

The formulations were prepared by milling the following three slurries in a 0.5 oz amber bottle containing 7.5 ml 0.8 mm Ytria-doped Zirconia media for 72 hours on a U.S. Stoneware roller mill:

- (1) 20% rapamycin/10% Plurionic F68
- (2) 20% rapamycin/5% Plurionic F68
- (3) 20% rapamycin/2.5% Plurionic F68

Following storage for two and half months, no SECO degradation product was detected in any of the samples. These results show that various dosage strengths of rapamycin can be used in nanoparticulate formulations without any impact on the increased chemical stability of the drug.

* * * *

It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention, provided they come within the scope of the appended claims and their equivalents.

Claims:

1. A method for chemically stabilizing an active agent comprising:
 - (a) identifying and selecting at least one active agent which is unstable under one or more environmental conditions, wherein the active agent is poorly soluble in at least one liquid medium; and
 - (b) formulating particles of the active agent into a stable nanoparticulate composition by a method comprising:
 - (1) combining the active agent particles which are unstable under one or more environmental conditions; and
 - (2) at least one non-crosslinked surface stabilizer, wherein after said combining the at least one non-crosslinked surface stabilizer, adsorbs to the surface of the active agent particles to produce a composition in which the active agent particles have an effective average particle size of less than about 2 microns;

wherein the resultant nanoparticulate active agent composition is stable under the one or more environmental conditions under which the active agent is unstable prior to formulating the active agent into a nanoparticulate composition.
2. The method of claim 1, wherein the at least one active agent is present in an amount selected from the group consisting of from about 99.5% to about 0.001%, from about 95% to about 0.1%, and from about 90% to about 0.5%, by weight, based on the total combined weight of the at least one active agent and at least one surface stabilizer, not including other excipients.
3. The method of claim 1 or 2, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from about 0.001 to about 99.5%, from about 0.1% to about 95%, and from about 0.5% to about 90%, by weight, based on the total combined weight of the at least one active agent and at least one surface stabilizer, not including other excipients.

4. The method of any of the previous claims, wherein after step (b) the active agent has an effective average particle size of less than about 1 micron.

5. The method of any of the previous claims, wherein after step (b) the active agent has an effective average particle size of less than about 600 nm.

6. The method of any of the previous claims, wherein after step (b) the active agent has an effective average particle size of less than about 500 nm.

7. The method of any of the previous claims, wherein after step (b) the active agent has an effective average particle size of less than about 400 nm.

8. The method of any of the previous claims, wherein after step (b) the active agent has an effective average particle size of less than about 300 nm.

9. The method of any of the previous claims, wherein after step (b) the active agent has an effective average particle size of less than about 200 nm.

10. The method of any of the previous claims, wherein after step (b) the active agent has an effective average particle size of less than about 100 nm.

11. The method of any of the previous claims, wherein after step (b) the active agent has an effective average particle size of less than about 50 nm.

12. The method of any of the previous claims, wherein the resultant composition is an injectable formulation.

13. The method of any of the previous claims, wherein the one or more environmental conditions are selected from the group consisting of exposure to a prolonged storage period, exposure to an elevated temperature, exposure to light, exposure to radiation, exposure to radiation causing photolysis, exposure to non-physiological pH, exposure to enzymes or other catalysts, exposure to water or other solvent molecules, exposure to oxidizing agents or other free radicals, and exposure to freezing-thawing temperature cycles.

14. The method of any of the previous claims, wherein the active agent is rapamycin.

15. The method of claim 14, wherein said rapamycin is stable following exposure to hydrolysis conditions.

16. The method of any of claims 1-13, wherein the active agent is paclitaxel.

17. The method of claim 16, wherein said paclitaxel is stable following exposure to basic pH conditions.

18. The method of any of the previous claims, wherein the active agent particles are in a crystalline phase, amorphous phase, semi-crystalline phase, semi-amorphous phase, or a combination thereof.

19. The method of any of the previous claims, wherein the surface stabilizer is selected from the group consisting of anionic, ionic, and cationic compounds.

The Degradation of Nanoparticulate Paclitaxel and Paclitaxel at 0.005 N NaOH

