# NANOSUSPENSIONS OF ANTI-RETROVIRAL AGENTS FOR INCREASED CENTRAL NERVOUS SYSTEM DELIVERY

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT:

Not Applicable.

## BACKGROUND OF THE INVENTION

### Technical Field

The present invention is directed to compositions comprising nanosuspensions of anti-retroviral agents and methods of their preparation. The compositions are prepared by a method of microprecipitation and energy addition. The compositions are particularly useful for delivering an anti-retroviral agent to the brain of a mammalian subject for the treatment of HIV infections.

### Background Art

Drugs or pharmaceutical agents that are used to treat a patient's brain disorders or diseases are usually administered orally. However, most of the ingested drug does not target the brain and is, instead, metabolized by the liver. This inefficient utilization of the drug may require ingestion of higher drug concentrations that can also be detrimental to the liver. Furthermore, lower amounts of drugs are able to reach the brain thereby requiring an increased frequency of doses taken by the patient. More efficient use of the drug can be realized both by eliminating liver metabolism and directly targeting the brain. One solution to this problem involves delivering a drug by using cells that are capable of reaching the brain to transport the drug. For example, one particular mode of delivery involves utilizing macrophages present in the patient's cerebrospinal fluid (CSF) to deliver drugs to the brain. This process requires that the pharmaceutical composition is in a particulate form that readily permits macrophage uptake by phagocytosis.

There are numerous advantages of drug delivery to the brain via macrophages over oral ingestion. The loading or amount of drug able to be delivered is increased because of high packing inherent in a particulate form that macrophages can phagocytise. Due to the

drug being administered to the CSF, liver metabolism is obviated because the drug is not exposed to the systemic circulation with consequent delivery to the liver. Once the drug is administered into the CSF, it can persist as an extended release depot for weeks or months.

As a particulate, the drug is taken up by brain macrophages which afford sanctuaries to viral and bacterial diseases such as the human immunodeficiency virus (HIV). Because the drug is concentrated in the brain macrophages, the infecting organism is exposed to much larger amounts of the drug thereby killing the organism. Macrophages can pass through the cerebrospinal fluid-brain barrier into the brain and release concentrations of the drug into the brain due to dissolution of the particle within the macrophages. As a result, free viral and bacterial organisms residing in the brain are exposed to the drug at concentrations higher than what is typically able to be delivered through oral administration. The brain is able to be more rapidly cleared of the microbial organisms thus preventing the emergence of drug-resistant organisms. Furthermore, the subsequent seeding and perpetuation within the body of the disease-causing organism within the body can be mitigated. Administering the drug in this manner allows increased utilization of the drug within the brain while permitting lower levels of drugs to be used. Excessive liver metabolism of drugs can be avoided and cost of therapy can be reduced through this invention.

There is needed, therefore, nanosuspension compositions of anti-retroviral agents, and methods of their manufacture, capable of delivery to the brain.

## SUMMARY OF THE INVENTION

The present invention provides compositions comprising nanosuspensions of anti-retroviral agents and methods of manufacture. The nanosuspensions are made by the process of microprecipitation and energy addition. Preferably, the nanosuspensions are made by the tandem process of microprecipitation-homogenization.

The nanosupensions of the present invention can deliver an anti-retroviral agent to the brain of a mammalian subject by cellular transport. The composition can be used to deliver the anti-retroviral agent to the brain to treat HIV infection. In a preferred embodiment, the process includes the steps of: (i) isolating cells from the mammalian subject, (ii) contacting the cells with a nanosuspension of anti-retroviral agent(s) particles having an average particle size of from about 100 nm to about 100 microns (preferably 100 nm to about 8 microns), (iii) allowing sufficient time for cell uptake of the particles, and (iv) administering to the

mammalian subject the loaded cells to deliver a portion of the pharmaceutical composition to the brain. There are numerous types of cells in the mammalian subject that are capable of this type of cellular uptake and transport of particles. These cells include, but are not limited to, T-lymphocytes, macrophages, monocytes, granulocytes, neutrophils, basophils, and eosinophils. The method can be used to deliver the anti-retroviral agent to the brain to treat HIV infection.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the results of stress testing of Indinavir nanosuspension using tests designed to assess long-term stability of the formulation

FIG. 2 shows the long term stability data for Indinavir nanosuspension produced using high-pressure homogenization

## DETAILED DESCRIPTION OF THE INVENTION

The present invention is susceptible of embodiments in many different forms. Preferred embodiments of the invention are disclosed with the understanding that the present disclosure is to be considered as exemplifications of the principles of the invention and are not intended to limit the broad aspects of the invention to the embodiments illustrated.

The present invention provides compositions comprising dispersions of anti-retroviral agents and methods of manufacture. The dispersions, or nanosuspensions, are made by the process of microprecipitation and energy addition. Preferably, the nanosuspensions are made by the tandem process of microprecipitation-homogenization.

The anti-retroviral agent in these processes can be a protease inhibitor, a nucleoside reverse transcriptase inhibitor, or a non-nucleoside reverse transcriptase inhibitor. Examples of protease inhibitors include but are not limited to indinavir, ritonavir, saquinavir, and nelfinavir. Examples of nucleoside reverse transcriptase inhibitors include but are not limited to zidovudine, didanosine, stavudine, zalcitabine, and lamivudine. Examples of non-nucleoside reverse transcriptase inhibitors include but are not limited to nevirapin and delaviradine.

The present invention provides a method for delivering a pharmaceutical composition to the brain of a mammalian subject through cellular transport. The following description of

the pharmaceutical composition applies to all embodiments of this invention. The pharmaceutical composition can be poorly water soluble or water soluble. The pharmaceutical composition can also be a therapeutic agent or a diagnostic agent. The therapeutic agents can include any compounds that are used to treat central nervous system disorders or brain diseases or disorders. The central nervous system disorders can be Parkinson's disease, Alzheimer's disease, cancer, viral infection, fungal infection, bacterial infection, and spongiform encephalopathy.

The pharmaceutical composition can further include a surfactant to stabilize the pharmaceutical composition. The surfactant can be selected from a variety of known anionic surfactants, cationic surfactants, nonionic surfactants and surface active biological modifiers.

Preferably the pharmaceutical composition is a poorly water-soluble compound. What is meant by "poorly water soluble" is a solubility of the compound in water of less than about 10 mg/mL, and preferably less than 1 mg/mL. These poorly water-soluble compounds are most suitable for aqueous suspension preparations since there are limited alternatives of formulating these compounds in an aqueous medium.

The following description of particles also applies to all embodiments of the present invention. The particles in the dispersion can be amorphous, semicrystalline, crystalline, or a combination thereof as determined by XRD. Prior to administration, the pharmaceutical composition can be homogenized through a homogenization process. The pharmaceutical composition can also be homogenized through a microprecipitation/homogenization process.

The dispersion of the pharmaceutical composition can be sterilized prior to administering. Sterilization can be performed by any medical sterilization process including heat sterilization or sterilization by gamma irradiation.

The present invention can be practiced with water-soluble compounds. These water soluble active compounds are entrapped in a solid carrier matrix (for example, polylactate-polyglycolate copolymer, albumin, starch), or encapsulated in a surrounding vesicle that is impermeable to the pharmaceutical compound. This encapsulating vesicle can be a polymeric coating such as polyacrylate. Further, the small particles prepared from these water soluble compounds can be modified to improve chemical stability and control the pharmacokinetic properties of the compounds by controlling the release of the compounds from the particles. Examples of water-soluble compounds include, but are not limited to, simple organic compounds, proteins, peptides, nucleotides, oligonucleotides, and carbohydrates.

The particles utilized in the present invention have an average effective particle size of generally from about 100 nm to about 100 μm, preferably from about 100 nm to about 8 microns, and most preferably from about 100 nm to about 400 nm, as measured by dynamic light scattering methods, e.g., photocorrelation spectroscopy, laser diffraction, low-angle laser light scattering (LALLS), medium-angle laser light scattering (MALLS), light obscuration methods (Coulter method, for example), rheology, or microscopy (light or electron). The preferred average effective particle size depends on factors such as the intended route of administration, formulation, solubility, toxicity and bioavailability of the compound.

A. Preparation of the pharmaceutical composition as particles

The processes for preparing the particles used in the present invention can be accomplished through numerous techniques known to those skilled in the art. A representative, but non-exhaustive, discussion of techniques for preparing particle dispersions of pharmaceutical compositions follows.

I. Energy Addition Techniques for Forming Small Particle Dispersions

In general, the method of preparing small particle dispersions using energy addition techniques includes the step of adding the pharmaceutically active compound, which sometimes shall be referred to as a drug, in bulk form to a suitable vehicle such as water or aqueous based solution containing one or more of the surfactants set forth below, or other liquid in which the pharmaceutical compound is not appreciably soluble, to form a first suspension. Energy is added to the first suspension to form a particle dispersion. Energy is added by mechanical grinding, pearl milling, ball milling, hammer milling, fluid energy milling or wet grinding. Such techniques are disclosed in U.S. Patent No. 5,145,684, which is incorporated herein by reference and made a part hereof.

Energy addition techniques further include subjecting the first suspension to high shear conditions including cavitation, shearing or impact forces utilizing a microfluidizer. The present invention further contemplates adding energy to the first suspension using a piston gap homogenizer or counter current flow homogenizer such as those disclosed in U.S. Patent No. 5,091,188 which is incorporated herein by reference and made a part hereof. Suitable piston gap homogenizers are commercially available under the product name EMULSIFLEX by Avestin, and French Pressure Cells sold by Spectronic Instruments. Suitable microfluidizers are available from Microfluidics Corp.

The step of adding energy can also be accomplished using sonication techniques. The step of sonicating can be carried out with any suitable sonication device such as the Branson Model S-450A or Cole-Parmer 500/750 Watt Model. Such devices are well known in the industry. Typically the sonication device has a sonication horn or probe that is inserted into the first suspension to emit sonic energy into the solution. The sonicating device, in a preferred form of the invention, is operated at a frequency of from about 1 kHz to about 90 kHz and more preferably from about 20 kHz to about 40 kHz or any range or combination of ranges therein. The probe sizes can vary and preferably is in distinct sizes such as ½ inch or ¼ inch or the like.

Regardless of the energy addition technique used, the dispersion of small particles must be sterilized prior to use. Sterilization can be accomplished using the high-pressure sterilization techniques described below.

II. Precipitation Methods for Preparing Submicron Sized Particle Dispersions

Small particle dispersions can also be prepared by well known precipitation techniques. The following is a description of examples of precipitation techniques.

Microprecipitation Methods

One example of a microprecipitation method is disclosed in U.S. Patent No. 5,780,062, which is incorporated herein by reference and made a part hereof. The '062 patent discloses an organic compound precipitation process including: (i) dissolving the organic compound in a water-miscible first solvent; (ii) preparing a solution of polymer and an amphiphile in an aqueous second solvent and in which second solvent the organic compound is substantially insoluble whereby a polymer/amphiphile complex is formed; and (iii) mixing the solutions from steps (i) and (ii) so as to cause precipitation of an aggregate of the organic compound and the polymer/amphiphile complex.

Another example of a suitable precipitation process is disclosed in co-pending and commonly assigned U.S. Serial Nos. 09/874,499; 09/874,799; 09/874,637; and 10/021,692, which are incorporated herein by reference and made a part hereof. The processes disclosed include the steps of: (1) dissolving an organic compound in a water miscible first organic solvent to create a first solution; (2) mixing the first solution with a second solvent or water to precipitate the organic compound to create a first suspension; and (3) adding energy to the first suspension in the form of high-shear mixing or heat to provide a dispersion of small

particles. One or more optional surface modifiers set forth below can be added to the first organic solvent or the second aqueous solution.

Emulsion Precipitation Methods

One suitable emulsion precipitation technique is disclosed in the co-pending and commonly assigned U.S. Serial No. 09/964,273, which is incorporated herein by reference and is made a part hereof. In this approach, the process includes the steps of: (1) providing a multiphase system having an organic phase and an aqueous phase, the organic phase having a pharmaceutically active compound therein; and (2) sonicating the system to evaporate a portion of the organic phase to cause precipitation of the compound in the aqueous phase to form a dispersion of small particles. The step of providing a multiphase system includes the steps of: (1) mixing a water immiscible solvent with the pharmaceutically active compound to define an organic solution, (2) preparing an aqueous based solution with one or more surface active compounds, and (3) mixing the organic solution with the aqueous solution to form the multiphase system. The step of mixing the organic phase and the aqueous phase can include the use of piston gap homogenizers, colloidal mills, high speed stirring equipment, extrusion equipment, manual agitation or shaking equipment, microfluidizer, or other equipment or techniques for providing high shear conditions. The crude emulsion will have oil droplets in the water of a size of approximately less than 1μm in diameter. The crude emulsion is sonicated to define a microemulsion and eventually to provide a dispersion of small particles.

Another approach to preparing a dispersion of small particles is disclosed in co-pending and commonly assigned U.S. Serial No. 10/183,035, which is incorporated herein by reference and made a part hereof. The process includes the steps of: (1) providing a crude dispersion of a multiphase system having an organic phase and an aqueous phase, the organic phase having a pharmaceutical compound therein; (2) providing energy to the crude dispersion to form a fine dispersion; (3) freezing the fine dispersion; and (4) lyophilizing the fine dispersion to obtain small particles of the pharmaceutical compound. The small particles can be sterilized by the techniques set forth below or the small particles can be reconsistuted in an aqueous medium and sterilized.

The step of providing a multiphase system includes the steps of: (1) mixing a water immiscible solvent with the pharmaceutically effective compound to define an organic solution; (2) preparing an aqueous based solution with one or more surface active

compounds; and (3) mixing the organic solution with the aqueous solution to form the multiphase system. The step of mixing the organic phase and the aqueous phase includes the use of piston gap homogenizers, colloidal mills, high speed stirring equipment, extrusion equipment, manual agitation or shaking equipment, microfluidizer, or other equipment or techniques for providing high shear conditions.

Solvent Anti-solvent Precipitation

Small particle dispersions can also be prepared using solvent anti-solvent precipitation technique disclosed in U.S. Patent Nos. 5,118,528 and 5,100,591 which are incorporated herein by reference and made a part hereof. The process includes the steps of: (1) preparing a liquid phase of a biologically active substance in a solvent or a mixture of solvents to which may be added one or more surfactants; (2) preparing a second liquid phase of a non-solvent or a mixture of non-solvents, the non-solvent is miscible with the solvent or mixture of solvents for the substance; (3) adding together the solutions of (1) and (2) with stirring; and (4) removing of unwanted solvents to produce a dispersion of small particles

Phase Inversion Precipitation

Small particle dispersions can be formed using phase inversion precipitation as disclosed in U.S. Patent Nos. 6,235,224, 6,143,211 and U.S. Patent Application No. 2001/0042932, each of which is incorporated herein by reference and made a part hereof. Phase inversion is a term used to describe the physical phenomena by which a polymer dissolved in a continuous phase solvent system inverts into a solid macromolecular network in which the polymer is the continuous phase. One method to induce phase inversion is by the addition of a nonsolvent to the continuous phase. The polymer undergoes a transition from a single phase to an unstable two phase mixture: polymer rich and polymer poor fractions. Micellar droplets of nonsolvent in the polymer rich phase serve as nucleation sites and become coated with polymer. The '224 patent discloses that phase inversion of polymer solutions under certain conditions can bring about spontaneous formation of discrete microparticles, including nanoparticles. The '224 patent discloses dissolving or dispersing a polymer in a solvent. A pharmaceutical agent is also dissolved or dispersed in the solvent. For the crystal seeding step to be effective in this process it is desirable the agent is dissolved in the solvent. The polymer, the agent and the solvent together form a mixture having a continuous phase, wherein the solvent is the continuous phase. The mixture is then introduced into at least tenfold excess of a miscible nonsolvent to cause the spontaneous

formation of the microencapsulated microparticles of the agent having an average particle size of between 10 nm and 10μm. The particle size is influenced by the solvent:nonsolvent volume ratio, polymer concentration, the viscosity of the polymer-solvent solution, the molecular weight of the polymer, and the characteristics of the solvent-nonsolvent pair.

pH Shift Precipitation

Small particle dispersions can be formed by pH shift precipitation techniques. Such techniques typically include a step of dissolving a drug in a solution having a pH where the drug is soluble, followed by the step of changing the pH to a point where the drug is no longer soluble. The pH can be acidic or basic, depending on the particular pharmaceutical compound. The solution is then neutralized to form a dispersion of small particles. One suitable pH shifting precipitation process is disclosed in U.S. Patent No. 5,665,331, which is incorporated herein by reference and made a part hereof. The process includes the step of dissolving of the pharmaceutical agent together with a crystal growth modifier (CGM) in an alkaline solution and then neutralizing the solution with an acid in the presence of suitable surface-modifying surface-active agent or agents to form a small particle dispersion of the pharmaceutical agent. The precipitation step can be followed by steps of diafiltration clean-up of the dispersion and then adjusting the concentration of the dispersion to a desired level.

Other examples of pH shifting precipitation methods are disclosed in U.S. Patent Nos. 5,716,642; 5,662,883; 5,560,932; and 4,608,278, which are incorporated herein by reference and are made a part hereof.

Infusion Precipitation Method

Suitable infusion precipitation techniques to form small particle dispersions are disclosed in the U.S. Patent Nos. 4,997,454 and 4,826,689, which are incorporated herein by reference and made a part hereof. First, a suitable solid compound is dissolved in a suitable organic solvent to form a solvent mixture. Then, a precipitating nonsolvent miscible with the organic solvent is infused into the solvent mixture at a temperature between about -10°C and about 100°C and at an infusion rate of from about 0.01 ml per minute to about 1000 ml per minute per volume of 50 ml to produce a suspension of precipitated non-aggregated solid particles of the compound with a substantially uniform mean diameter of less than 10μm. Agitation (e.g., by stirring) of the solution being infused with the precipitating nonsolvent is preferred. The nonsolvent may contain a surfactant to stabilize the particles against

aggregation. The particles are then separated from the solvent. Depending on the solid compound and the desired particle size, the parameters of temperature, ratio of nonsolvent to solvent, infusion rate, stir rate, and volume can be varied according to the invention. The particle size is proportional to the ratio of nonsolvent:solvent volumes and the temperature of infusion and is inversely proportional to the infusion rate and the stirring rate. The precipitating nonsolvent may be aqueous or non-aqueous, depending upon the relative solubility of the compound and the desired suspending vehicle.

Temperature Shift Precipitation

Temperature shift precipitation techniques may also be used to form small particle dispersions. This technique is disclosed in U.S. Patent No. 5,188,837, which is incorporated herein by reference and made a part hereof. In an embodiment of the invention, lipospheres are prepared by the steps of: (1) melting or dissolving a substance such as a drug to be delivered in a molten vehicle to form a liquid of the substance to be delivered; (2) adding a phospholipid along with an aqueous medium to the melted substance or vehicle at a temperature higher than the melting temperature of the substance or vehicle; (3) mixing the suspension at a temperature above the melting temperature of the vehicle until a homogenous fine preparation is obtained; and then (4) rapidly cooling the preparation to room temperature or below.

Solvent Evaporation Precipitation

Solvent evaporation precipitation techniques are disclosed in U.S. Patent No. 4,973,465 which is incorporated herein by reference and made a part hereof. The '465 Patent discloses methods for preparing microcrystals including the steps of: (1) providing a solution of a pharmaceutical composition and a phospholipid dissolved in a common organic solvent or combination of solvents, (2) evaporating the solvent or solvents and (3) suspending the film obtained by evaporation of the solvent or solvents in an aqueous solution by vigorous stirring to form a dispersion of small particles. The solvent can be removed by adding energy to the solution to evaporate a sufficient quantity of the solvent to cause precipitation of the compound. The solvent can also be removed by other well known techniques such as applying a vacuum to the solution or blowing nitrogen over the solution.

Reaction Precipitation

Reaction precipitation includes the steps of dissolving the pharmaceutical compound into a suitable solvent to form a solution. The compound should be added in an amount at or below the saturation point of the compound in the solvent. The compound is modified by reacting with a chemical agent or by modification in response to adding energy such as heat or UV light or the like such that the modified compound has a lower solubility in the solvent and precipitates from the solution to form a small particle dispersion.

Compressed Fluid Precipitation

A suitable technique for precipitating by compressed fluid is disclosed in WO 97/14407 to Johnston, which is incorporated herein by reference and made a part hereof. The method includes the steps of dissolving a water-insoluble drug in a solvent to form a solution. The solution is then sprayed into a compressed fluid, which can be a gas, liquid or supercritical fluid. The addition of the compressed fluid to a solution of a solute in a solvent causes the solute to attain or approach supersaturated state and to precipitate out as fine particles. In this case, the compressed fluid acts as an anti-solvent which lowers the cohesive energy density of the solvent in which the drug is dissolved.

Alternatively, the drug can be dissolved in the compressed fluid which is then sprayed into an aqueous phase. The rapid expansion of the compressed fluid reduces the solvent power of the fluid, which in turn causes the solute to precipitate out as small particles in the aqueous phase. In this case, the compressed fluid acts as a solvent.

In order to stabilize the particles against aggregation, a surface modifier, such as a surfactant, is included in this technique.

Protein Microsphere Precipitation

Microspheres or microparticles utilized in this invention can also be produced from a process involving mixing or dissolving macromolecules such as proteins with a water soluble polymer. This process is disclosed in U.S. Patent Nos. 5,849,884, 5,981,719, 6,090,925, 6,268,053, 6,458,387, and U.S. Provisional Application No. 60/244,098, which are incorporated herein by reference and made a part hereof. In an embodiment of the invention, microspheres are prepared by mixing a macromolecule in solution with a polymer or a mixture of polymers in solution at a pH near the isoelectric point of the macromolecule. The mixture is incubated in the presence of an energy source, such as heat, radiation, or

ionization, for a predetermined amount of time. The resulting microspheres can be removed from any unincorporated components present in the solution by physical separation methods.

There are numerous other methodologies for preparing small particle dispersions. The present invention provides a methodology for terminally sterilizing such dispersions without significantly impacting the efficacy of the preparation.

III. Additional methods for preparing particle dispersions of pharmaceutical compositions

The following additional processes for preparing particles of pharmaceutical compositions (i.e. organic compound) used in the present invention can be separated into four general categories. Each of the categories of processes share the steps of: (1) dissolving an organic compound in a water miscible first solvent to create a first solution, (2) mixing the first solution with a second solvent of water to precipitate the organic compound to create a pre-suspension, and (3) adding energy to the first suspension in the form of high-shear mixing or heat, or a combination of both, to provide a stable form of the organic compound having the desired size ranges defined above. The mixing steps and the adding energy step can be carried out in consecutive steps or simultaneously.

The categories of processes are distinguished based upon the physical properties of the organic compound as determined through x-ray diffraction studies, differential scanning calorimetry (DSC) studies, or other suitable study conducted prior to the energy-addition step and after the energy-addition step. In the first process category, prior to the energy-addition step the organic compound in the first suspension takes an amorphous form, a semi-crystalline form or a supercooled liquid form and has an average effective particle size. After the energy-addition step the organic compound is in a crystalline form having an average effective particle size essentially the same or less than that of the first suspension.

In the second process category, prior to the energy-addition step the organic compound is in a crystalline form and has an average effective particle size. After the energy-addition step the organic compound is in a crystalline form having essentially the same average effective particle size as prior to the energy-addition step but the crystals after the energy-addition step are less likely to aggregate.

The lower tendency of the organic compound to aggregate is observed by laser dynamic light scattering and light microscopy.

In the third process category, prior to the energy-addition step the organic compound is in a crystalline form that is friable and has an average effective particle size. What is

meant by the term "friable" is that the particles are fragile and are more easily broken down into smaller particles. After the energy-addition step the organic compound is in a crystalline form having an average effective particle size smaller than the crystals of the pre-suspension. By taking the steps necessary to place the organic compound in a crystalline form that is friable, the subsequent energy-addition step can be carried out more quickly and efficiently when compared to an organic compound in a less friable crystalline morphology.

In the fourth process category, the first solution and second solvent are simultaneously subjected to the energy-addition step. Thus, the physical properties of the organic compound before and after the energy addition step were not measured.

The energy-addition step can be carried out in any fashion wherein the first suspension or the first solution and second solvent are exposed to cavitation, shearing or impact forces. In one preferred form, the energy-addition step is an annealing step. Annealing is defined in this invention as the process of converting matter that is thermodynamically unstable into a more stable form by single or repeated application of energy (direct heat or mechanical stress), followed by thermal relaxation. This lowering of energy may be achieved by conversion of the solid form from a less ordered to a more ordered lattice structure. Alternatively, this stabilization may occur by a reordering of the surfactant molecules at the solid-liquid interface.

These four process categories are shown separately below. It should be understood, however, that the process conditions such as choice of surfactants or combination of surfactants, amount of surfactant used, temperature of reaction, rate of mixing of solutions, rate of precipitation and the like can be selected to allow for any drug to be processed under any one of the categories discussed next.

The first process category, as well as the second, third, and fourth process categories, can be further divided into two subcategories, Method A and B.

The first solvent according to the following processes is a solvent or mixture of solvents in which the organic compound of interest is relatively soluble and which is miscible with the second solvent. Such solvents include, but are not limited to water-miscible protic compounds, in which a hydrogen atom in the molecule is bound to an electronegative atom such as oxygen, nitrogen, or other Group VA, VIA and VII A in the Periodic Table of elements. Examples of such solvents include, but are not limited to, alcohols, amines (primary or secondary), oximes, hydroxamic acids, carboxylic acids, sulfonic acids, phosphonic acids, phosphoric acids, amides and ureas.

Other examples of the first solvent also include aprotic organic solvents. Some of these aprotic solvents can form hydrogen bonds with water, but can only act as proton acceptors because they lack effective proton donating groups. One class of aprotic solvents is a dipolar aprotic solvent, as defined by the International Union of Pure and Applied Chemistry (IUPAC Compendium of Chemical Terminology, 2nd Ed., 1997):

> A solvent with a comparatively high relative permittivity (or dielectric constant), greater than ca. 15, and a sizable permanent dipole moment, that cannot donate suitably labile hydrogen atoms to form strong hydrogen bonds, e.g. dimethyl sulfoxide.

Dipolar aprotic solvents can be selected from the group consisting of: amides (fully substituted, with nitrogen lacking attached hydrogen atoms), ureas (fully substituted, with no hydrogen atoms attached to nitrogen), ethers, cyclic ethers, nitriles, ketones, sulfones, sulfoxides, fully substituted phosphates, phosphonate esters, phosphoramides, nitro compounds, and the like. Dimethylsulfoxide (DMSO), N-methyl-2-pyrrolidinone (NMP), 2-pyrrolidinone, 1,3-dimethylimidazolidinone (DMI), dimethylacetamide (DMA), dimethylformamide (DMF), dioxane, acetone, tetrahydrofuran (THF), tetramethylenesulfone (sulfolane), acetonitrile, and hexamethylphosphoramide (HMPA), nitromethane, among others, are members of this class.

Solvents may also be chosen that are generally water-immiscible, but have sufficient water solubility at low volumes (less than 10%) to act as a water-miscible first solvent at these reduced volumes. Examples include aromatic hydrocarbons, alkenes, alkanes, and halogenated aromatics, halogenated alkenes and halogenated alkanes. Aromatics include, but are not limited to, benzene (substituted or unsubstituted), and monocyclic or polycyclic arenes. Examples of substituted benzenes include, but are not limited to, xylenes (ortho, meta, or para), and toluene. Examples of alkanes include but are not limited to hexane, neopentane, heptane, isooctane, and cyclohexane. Examples of halogenated aromatics include, but are not restricted to, chlorobenzene, bromobenzene, and chlorotoluene. Examples of halogenated alkanes and alkenes include, but are not restricted to, trichloroethane, methylene chloride, ethylenedichloride (EDC), and the like.

Examples of the all of the above solvent classes include but are not limited to: N-methyl-2-pyrrolidinone (also called N-methyl-2-pyrrolidone), 2-pyrrolidinone (also called 2-pyrrolidone), 1,3-dimethyl-2-imidazolidinone (DMI), dimethylsulfoxide, dimethylacetamide,

acetic acid, lactic acid, methanol, ethanol, isopropanol, 3-pentanol, n-propanol, benzyl alcohol, glycerol, butylene glycol (butanediol), ethylene glycol, propylene glycol, mono- and diacylated monoglycerides (such as glyceryl caprylate), dimethyl isosorbide, acetone, dimethylsulfone, dimethylformamide, 1,4-dioxane, tetramethylenesulfone (sulfolane), acetonitrile, nitromethane, tetramethylurea, hexamethylphosphoramide (HMPA), tetrahydrofuran (THF), dioxane, diethylether, tert-butylmethyl ether (TBME), aromatic hydrocarbons, alkenes, alkanes, halogenated aromatics, halogenated alkenes, halogenated alkanes, xylene, toluene, benzene, substituted benzene, ethyl acetate, methyl acetate, butyl acetate, chlorobenzene, bromobenzene, chlorotoluene, trichloroethane, methylene chloride, ethylenedichloride (EDC), hexane, neopentane, heptane, isooctane, cyclohexane, polyethylene glycol (PEG, for example, PEG-4, PEG-8, PEG-9, PEG-12, PEG-14, PEG-16, PEG-120, PEG-75, PEG-150), polyethylene glycol esters (examples such as PEG-4 dilaurate, PEG-20 dilaurate, PEG-6 isostearate, PEG-8 palmitostearate, PEG-150 palmitostearate), polyethylene glycol sorbitans (such as PEG-20 sorbitan isostearate), polyethylene glycol monoalkyl ethers (examples such as PEG-3 dimethyl ether, PEG-4 dimethyl ether), polypropylene glycol (PPG), polypropylene alginate, PPG-10 butanediol, PPG-10 methyl glucose ether, PPG-20 methyl glucose ether, PPG-15 stearyl ether, propylene glycol dicaprylate/dicaprate, propylene glycol laurate, and glycofurol (tetrahydrofurfuryl alcohol polyethylene glycol ether). A preferred first solvent is N-methyl-2-pyrrolidinone. Another preferred first solvent is lactic acid.

The second solvent is an aqueous solvent. This aqueous solvent may be water by itself. This solvent may also contain buffers, salts, surfactant(s), water-soluble polymers, and combinations of these excipients.

Method A

In Method A (see FIG. 1), the organic compound ("drug") is first dissolved in the first solvent to create a first solution. The organic compound can be added from about 0.1% (w/v) to about 50% (w/v) depending on the solubility of the organic compound in the first solvent. Heating of the concentrate from about 30°C to about 100°C may be necessary to ensure total dissolution of the compound in the first solvent.

A second aqueous solvent is provided with one or more optional surface modifiers such as an anionic surfactant, a cationic surfactant, a nonionic surfactant or a biologically surface active molecule added thereto. Suitable anionic surfactants include but are not

limited to alkyl sulfonates, alkyl phosphates, alkyl phosphonates, potassium laurate, triethanolamine stearate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl inosine, phosphatidylserine, phosphatidic acid and their salts, glyceryl esters, sodium carboxymethylcellulose, cholic acid and other bile acids (e.g., cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid) and salts thereof (e.g., sodium deoxycholate, etc.). Suitable cationic surfactants include but are not limited to quaternary ammonium compounds, such as benzalkonium chloride, cetyltrimethylammonium bromide, chitosans, lauryldimethylbenzylammonium chloride, acyl carnitine hydrochlorides, or alkyl pyridinium halides. As anionic surfactants, phospholipids may be used. Suitable phospholipids include, for example phosphatidylcholine, phosphatidylethanolamine, diacyl-glycero-phosphoethanolamine (such as dimyristoyl-glycero-phosphoethanolamine (DMPE), dipalmitoyl-glycero-phosphoethanolamine (DPPE), distearoyl-glycero-phosphoethanolamine (DSPE), and dioleolyl-glycero-phosphoethanolamine (DOPE)), phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, lysophospholipids, egg or soybean phospholipid or a combination thereof. The phospholipid may be salted or desalted, hydrogenated or partially hydrogenated or natural semisynthetic or synthetic. The phospholipid may also be conjugated with a water-soluble or hydrophilic polymer. A preferred polymer is polyethylene glycol (PEG), which is also known as the monomethoxy polyethyleneglycol (mPEG). The molecule weights of the PEG can vary, for example, from 200 to 50,000. Some commonly used PEG's that are commercially available include PEG 350, PEG 550, PEG 750, PEG 1000, PEG 2000, PEG 3000, and PEG 5000. The phospholipid or the PEG-phospholipid conjugate may also incorporate a functional group which can covalently attach to a ligand including but not limited to proteins, peptides, carbohydrates, glycoproteins, antibodies, or pharmaceutically active agents. These functional groups may conjugate with the ligands through, for example, amide bond formation, disulfide or thioether formation, or biotin/streptavidin binding. Examples of the ligand-binding functional groups include but are not limited to hexanoylamine, dodecanylamine, 1,12-dodecanedicarboxylate, thioethanol, 4-(p-maleimidophenyl)butyramide (MPB), 4-(p-maleimidomethyl)cyclohexane-carboxamide (MCC), 3-(2-pyridyldithio)propionate (PDP), succinate, glutarate, dodecanoate, and biotin.

Suitable nonionic surfactants include: polyoxyethylene fatty alcohol ethers (Macrogol and Brij), polyoxyethylene sorbitan fatty acid esters (Polysorbates), polyoxyethylene fatty

acid esters (Myrj), sorbitan esters (Span), glycerol monostearate, polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers (poloxamers), poloxamines, methylcellulose, hydroxymethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, noncrystalline cellulose, polysaccharides including starch and starch derivatives such as hydroxyethylstarch (HES), polyvinyl alcohol, and polyvinylpyrrolidone. In a preferred form, the nonionic surfactant is a polyoxyethylene and polyoxypropylene copolymer and preferably a block copolymer of propylene glycol and ethylene glycol. Such polymers are sold under the tradename POLOXAMER also sometimes referred to as PLURONIC®, and sold by several suppliers including Spectrum Chemical and Ruger. Among polyoxyethylene fatty acid esters is included those having short alkyl chains. One example of such a surfactant is SOLUTOL® HS 15, polyethylene-660-hydroxystearate, manufactured by BASF Aktiengesellschaft.

Surface-active biological molecules include such molecules as albumin, casein, hirudin or other appropriate proteins. Polysaccharide biologics are also included, and consist of but not limited to, starches, heparin and chitosans.

It may also be desirable to add a pH adjusting agent to the second solvent such as sodium hydroxide, hydrochloric acid, tris buffer or citrate, acetate, lactate, meglumine, or the like. The second solvent should have a pH within the range of from about 3 to about 11.

For oral dosage forms one or more of the following excipients may be utilized: gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens™, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP). Most of these excipients are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986. The surface modifiers are commercially available and/or can be

prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

In a preferred form, the method for preparing small particles of an organic compound includes the steps of adding the first solution to the second solvent. The addition rate is dependent on the batch size, and precipitation kinetics for the organic compound. Typically, for a small-scale laboratory process (preparation of 1 liter), the addition rate is from about 0.05 cc per minute to about 10 cc per minute. During the addition, the solutions should be under constant agitation. It has been observed using light microscopy that amorphous particles, semi-crystalline solids, or a supercooled liquid are formed to create a pre-suspension. The method further includes the step of subjecting the pre-suspension to an energy-addition step to convert the amorphous particles, supercooled liquid or semicrystalline solid to a more stable, crystalline solid state. The resulting particles will have an average effective particles size as measured by dynamic light scattering methods (e.g., photocorrelation spectroscopy, laser diffraction, low-angle laser light scattering (LALLS), medium-angle laser light scattering (MALLS), light obscuration methods (Coulter method, for example), rheology, or microscopy (light or electron) within the ranges set forth above). In process category four, the first solution and the second solvent are combined while simultaneously conducting the energy-addition step.

The energy-addition step involves adding energy through sonication, homogenization, countercurrent flow homogenization, microfluidization, or other methods of providing impact, shear or cavitation forces. The sample may be cooled or heated during this stage. In one preferred form, the energy-addition step is effected by a piston gap homogenizer such as the one sold by Avestin Inc. under the product designation EmulsiFlex-C160. In another preferred form, the energy-addition step may be accomplished by ultrasonication using an ultrasonic processor such as the Vibra-Cell Ultrasonic Processor (600W), manufactured by Sonics and Materials, Inc. In yet another preferred form, the energy-addition step may be accomplished by use of an emulsification apparatus as described in U.S. Patent No. 5,720,551 which is incorporated herein by reference and made a part hereof.

Depending upon the rate of energy addition, it may be desirable to adjust the temperature of the processed sample to within the range of from approximately –30°C to 30°C. Alternatively, in order to effect a desired phase change in the processed solid, it may also be necessary to heat the pre-suspension to a temperature within the range of from about 30°C to about 100°C during the energy-addition step.

Method B

Method B differs from Method A in the following respects. The first difference is a surfactant or combination of surfactants is added to the first solution. The surfactants may be selected from the groups of anionic, nonionic, cationic surfactants, and surface-active biological modifiers set forth above.

Comparative Example of Method A and Method B and USPN 5,780,062

United States Patent No. 5,780,062 discloses a process for preparing small particles of an organic compound by first dissolving the compound in a suitable water-miscible first solvent. A second solution is prepared by dissolving a polymer and an amphiphile in aqueous solvent. The first solution is then added to the second solution to form a precipitate that consists of the organic compound and a polymer-amphiphile complex. The '062 Patent does not disclose utilizing the energy-addition step of this process in Methods A and B. Lack of stability is typically evidenced by rapid aggregation and particle growth. In some instances, amorphous particles recrystallize as large crystals. Adding energy to the pre-suspension in the manner disclosed above typically affords particles that show decreased rates of particle aggregation and growth, as well as the absence of recrystallization upon product storage.

Methods A and B are further distinguished from the process of the '062 patent by the absence of a step of forming a polymer-amphiphile complex prior to precipitation. In Method A, such a complex cannot be formed as no polymer is added to the diluent (aqueous) phase. In Method B, the surfactant, which may also act as an amphiphile, or polymer, is dissolved with the organic compound in the first solvent. This precludes the formation of any amphiphile-polymer complexes prior to precipitation. In the '062 Patent, successful precipitation of small particles relies upon the formation of an amphiphile-polymer complex prior to precipitation. The '062 Patent discloses the amphiphile-polymer complex forms aggregates in the aqueous second solution. The '062 Patent explains the hydrophobic organic compound interacts with the amphiphile-polymer complex, thereby reducing solubility of these aggregates and causing precipitation. In the present process, it has been demonstrated that the inclusion of the surfactant or polymer in the first solvent (Method B) leads, upon subsequent addition to second solvent, to formation of a more uniform, finer particulate than is afforded by the process outlined by the '062 Patent.

To this end, two formulations were prepared and analyzed. Each of the formulations has two solutions, a concentrate and an aqueous diluent, which are mixed together and then

sonicated. The concentrate in each formulation has an organic compound (itraconazole), a water miscible solvent (N-methyl-2-pyrrolidinone or NMP) and possibly a polymer (poloxamer 188). The aqueous diluent has water, a tris buffer and possibly a polymer (poloxamer 188) and/or a surfactant (sodium deoxycholate). The average particle diameter of the organic particle is measured prior to sonication and after sonication.

The first formulation A has as the concentrate itraconazole and NMP. The aqueous diluent includes water, poloxamer 188, tris buffer and sodium deoxycholate. Thus the aqueous diluent includes a polymer (poloxamer 188), and an amphiphile (sodium deoxycholate), which may form a polymer/amphiphile complex, and, therefore, is in accordance with the disclosure of the '062 Patent. (However, again the '062 Patent does not disclose an energy addition step.)

The second formulation B has as the concentrate itraconazole, NMP and poloxamer 188. The aqueous diluent includes water, tris buffer and sodium deoxycholate. This formulation is made in accordance with the present process. Since the aqueous diluent does not contain a combination of a polymer (poloxamer) and an amphiphile (sodium deoxycholate), a polymer/amphiphile complex cannot form prior to the mixing step.

Table 1 shows the average particle diameters measured by laser diffraction on three replicate suspension preparations. An initial size determination was made, after which the sample was sonicated for 1 minute. The size determination was then repeated. The large size reduction upon sonication of Method A was indicative of particle aggregation.

Table 1:

| Method | Concentrate | Aqueous Diluent | Average particle diameter (microns) | After sonication (1 minute) |
|---|---|---|---|---|
| A | itraconazole (18%),N-methyl-2-pyrrolidinone (6 mL) | poloxamer 188 (2.3%),sodium deoxycholate (0.3%)tris buffer (5 mM, pH 8)water (qs to 94 mL) | 18.7<br>10.7<br>12.1 | 2.36<br>2.46<br>1.93 |
| B | itraconazole (18%)poloxamer 188 (37%)N-methyl-2-pyrrolidinone (6 mL) | sodium deoxycholate (0.3%)tris buffer (5 mM, pH 8)water (qs to 94 mL) | 0.194<br>0.178<br>0.181 | 0.198<br>0.179<br>0.177 |

A drug suspension resulting from application of the processes may be administered directly as an injectable solution, provided Water for Injection is used in formulation and an appropriate means for solution sterilization is applied. Sterilization may be accomplished by methods well known in the art such as steam or heat sterilization, gamma irradiation and the

like. Other sterilization methods, especially for particles in which greater than 99% of the particles are less than 200 nm, would also include pre-filtration first through a 3.0 micron filter followed by filtration through a 0.45-micron particle filter, followed by steam or heat sterilization or sterile filtration through two redundant 0.2-micron membrane filters. Yet another means of sterilization is sterile filtration of the concentrate prepared from the first solvent containing drug and optional surfactant or surfactants and sterile filtration of the aqueous diluent. These are then combined in a sterile mixing container, preferably in an isolated, sterile environment. Mixing, homogenization, and further processing of the suspension are then carried out under aseptic conditions.

Yet another procedure for sterilization would consist of heat sterilization or autoclaving within the homogenizer itself, before, during, or subsequent to the homogenization step. Processing after this heat treatment would be carried out under aseptic conditions.

Optionally, a solvent-free suspension may be produced by solvent removal after precipitation. This can be accomplished by centrifugation, dialysis, diafiltration, force-field fractionation, high-pressure filtration, reverse osmosis, or other separation techniques well known in the art. Complete removal of N-methyl-2-pyrrolidinone was typically carried out by one to three successive centrifugation runs; after each centrifugation (18,000 rpm for 30 minutes) the supernatant was decanted and discarded. A fresh volume of the suspension vehicle without the organic solvent was added to the remaining solids and the mixture was dispersed by homogenization. It will be recognized by those skilled in the art that other high-shear mixing techniques could be applied in this reconstitution step. Alternatively, the solvent-free particles can be formulated into various dosage forms as desired for a variety of administrative routes, such as oral, pulmonary, nasal, topical, intramuscular, and the like.

Furthermore, any undesired excipients such as surfactants may be replaced by a more desirable excipient by use of the separation methods described in the above paragraph. The solvent and first excipient may be discarded with the supernatant after centrifugation or filtration. A fresh volume of the suspension vehicle without the solvent and without the first excipient may then be added. Alternatively, a new surfactant may be added. For example, a suspension consisting of drug, N-methyl-2-pyrrolidinone (solvent), poloxamer 188 (first excipient), sodium deoxycholate, glycerol and water may be replaced with phospholipids (new surfactant), glycerol and water after centrifugation and removal of the supernatant.

I. First Process Category

The methods of the first process category generally include the step of dissolving the organic compound in a water miscible first solvent followed by the step of mixing this solution with an aqueous solvent to form a first suspension wherein the organic compound is in an amorphous form, a semicrystalline form or in a supercooled liquid form as determined by x-ray diffraction studies, DSC, light microscopy or other analytical techniques and has an average effective particle size within one of the effective particle size ranges set forth above. The mixing step is followed by an energy-addition step.

II. Second Process Category

The methods of the second processes category include essentially the same steps as in the steps of the first processes category but differ in the following respect. An x-ray diffraction, DSC or other suitable analytical techniques of the first suspension shows the organic compound in a crystalline form and having an average effective particle size. The organic compound after the energy-addition step has essentially the same average effective particle size as prior to the energy-addition step but has less of a tendency to aggregate into larger particles when compared to that of the particles of the first suspension. Without being bound to a theory, it is believed the differences in the particle stability may be due to a reordering of the surfactant molecules at the solid-liquid interface.

III. Third Process Category

The methods of the third category modify the first two steps of those of the first and second processes categories to ensure the organic compound in the first suspension is in a friable form having an average effective particle size (e.g., such as slender needles and thin plates). Friable particles can be formed by selecting suitable solvents, surfactants or combination of surfactants, the temperature of the individual solutions, the rate of mixing and rate of precipitation and the like. Friability may also be enhanced by the introduction of lattice defects (e.g., cleavage planes) during the steps of mixing the first solution with the aqueous solvent. This would arise by rapid crystallization such as that afforded in the precipitation step. In the energy-addition step these friable crystals are converted to crystals that are kinetically stabilized and having an average effective particle size smaller than those of the first suspension. Kinetically stabilized means particles have a reduced tendency to aggregate when compared to particles that are not kinetically stabilized. In such instance the energy-addition step results in a breaking up of the friable particles. By ensuring the particles

of the first suspension are in a friable state, the organic compound can more easily and more quickly be prepared into a particle within the desired size ranges when compared to processing an organic compound where the steps have not been taken to render it in a friable form.

IV. Fourth Process Category

The methods of the fourth process category include the steps of the first process category except that the mixing step is carried out simultaneously with the energy-addition step.

Polymorph Control

The present process further provides additional steps for controlling the crystal structure of an organic compound to ultimately produce a suspension of the compound in the desired size range and a desired crystal structure. What is meant by the term "crystal structure" is the arrangement of the atoms within the unit cell of the crystal. Compounds that can be crystallized into different crystal structures are said to be polymorphic. Identification of polymorphs is important step in drug formulation since different polymorphs of the same drug can show differences in solubility, therapeutic activity, bioavailability, and suspension stability. Accordingly, it is important to control the polymorphic form of the compound for ensuring product purity and batch-to-batch reproducibility.

The steps to control the polymorphic form of the compound includes seeding the first solution, the second solvent or the pre-suspension to ensure the formation of the desired polymorph. Seeding includes using a seed compound or adding energy. In a preferred form the seed compound is a pharmaceutically-active compound in the desired polymorphic form. Alternatively, the seed compound can also be an inert impurity, a compound unrelated in structure to the desired polymorph but with features that may lead to templating of a crystal nucleus, or an organic compound with a structure similar to that of the desired polymorph.

The seed compound can be precipitated from the first solution. This method includes the steps of adding the organic compound in sufficient quantity to exceed the solubility of the organic compound in the first solvent to create a supersaturated solution. The supersaturated solution is treated to precipitate the organic compound in the desired polymorphic form. Treating the supersaturated solution includes aging the solution for a time period until the formation of a crystal or crystals is observed to create a seeding mixture. It is also possible to add energy to the supersaturated solution to cause the organic compound to precipitate out of

the solution in the desired polymorph. The energy can be added in a variety of ways including the energy addition steps described above. Further energy can be added by heating, or by exposing the pre-suspension to electromagnetic energy, particle beam or electron beam sources. The electromagnetic energy includes light energy (ultraviolet, visible, or infrared) or coherent radiation such as that provided by a laser, microwave energy such as that provided by a maser (microwave amplification by stimulated emission of radiation), dynamic electromagnetic energy, or other radiation sources. It is further contemplated utilizing ultrasound, a static electric field, or a static magnetic field, or combinations of these, as the energy-addition source.

In a preferred form, the method for producing seed crystals from an aged supersaturated solution includes the steps of: (i) adding a quantity of an organic compound to the first organic solvent to create a supersaturated solution, (ii) aging the supersaturated solution to form detectable crystals to create a seeding mixture; and (iii) mixing the seeding mixture with the second solvent to precipitate the organic compound to create a pre-suspension. The first suspension can then be further processed as described in detail above to provide an aqueous suspension of the organic compound in the desired polymorph and in the desired size range.

Seeding can also be accomplished by adding energy to the first solution, the second solvent or the pre-suspension provided that the exposed liquid or liquids contain the organic compound or a seed material. The energy can be added in the same fashion as described above for the supersaturated solution.

Accordingly, the present processes utilize a composition of matter of an organic compound in a desired polymorphic form essentially free of the unspecified polymorph or polymorphs. In a preferred form, the organic compound is a pharmaceutically active substance. It is contemplated the methods described herein can be used to selectively produce a desired polymorph for numerous pharmaceutically active compounds.

B. Brain Targeting

Compositions of the present invention are particularly useful for delivering antiretroviral agents to the brain. Preferred methods of using the present invention compositions comprise the steps of: (i) providing a dispersion of a pharmaceutically effective antiretroviral agent in particle form, (ii) contacting the dispersion with cells for cell uptake to

form loaded cells, and (iii) administering the loaded cells for delivery to the brain of a portion of the particles. The processes for drug delivery to the brain can be divided into ex vivo and in vivo categories depending on whether the dispersion is contacted with the cells outside or inside the mammalian subject.

The ex vivo process includes the steps of: (i) isolating cells from the mammalian subject, (ii) contacting the cells with a dispersion of the pharmaceutical composition as particles having an average particle size of from about 100 nm to about 100 microns (preferably from about 100 nm to about 8 microns), (iii) allowing sufficient time for cell uptake of a portion of the particles to form loaded cells, and (iv) administering to the mammalian subject the loaded cells to deliver a portion of the pharmaceutical composition to the brain. There are numerous types of cells in the mammalian subject that are capable of this type of cellular uptake and transport of particles. These cells include, but are not limited to, macrophages, monocytes, granulocytes, neutrophils, basophils, and eosinophils. Furthermore, particles in the size range of from about 100 nm to about 8 microns are more readily taken up by these phagocytic organisms.

Isolating macrophages from the mammalian subject can be performed by a cell separator. For instance, the Fenwal cell separator (Baxter Healthcare Corp., Deefield, IL) can be used to isolate various cells. Once isolated, the particulate pharmaceutical composition is contacted with the isolated cell sample and incubated for short period of time to allow for cell uptake of the particles. Up to an hour can be given to permit sufficient cell uptake of the drug particles. Uptake by the cells of the dispersion of the pharmaceutical composition as particles may include phagocytosis or adsorption of the particle onto the surface of the cells. Furthermore, in a preferred form of the invention, the particles during contact with the cells are at a concentration higher than the thermodynamic or apparent solubility thereby allowing the particles to remain in particulate form during uptake and delivery to the brain by the cells.

For marginally soluble drugs, e.g. indinavir, the ex vivo procedure can be utilized provided that the isolated cells are able to phagocytize the pharmaceutical composition particles at a faster rate than the competing dissolution process. The particles should be large enough to allow for the cells to phagocytize the particles and deliver them to the brain before complete dissolution of the particle. Furthermore, the concentration of the pharmaceutical composition should be kept higher than the thermodynamic or apparent solubility of the composition so that the particle is able to remain in the crystalline state during phagocytosis.

The loaded cells can be administered intrathecally, epidurally, or through any procedure that can be used for delivery of medicine into the central nervous system. The loaded cells can also be administered into the vascular system of the mammalian subject, including administration into the veinous system or via the carotid artery. The step of administering can be by bolus injection or by continuous administration.

In another preferred embodiment, the pharmaceutical composition as particles is administered directly into the central nervous system of the mammalian subject, particularly the cerebrospinal fluid (CSF). The particles are of a sufficient size where they are engulfed by phagocytic cells residing in the CSF and transported past the cerebrospinal fluid-brain barrier (CFBB) into the brain. The particles may also be adsorbed onto the surface of these cells. Ordinarily, the CFBB acts to prevent entry of drugs into the brain. This invention exploits the use of these phagocytic cells as drug delivery vessels, particularly when the brain has an increase in the rate that macrophages will pass through the CFBB. In a preferred form of the invention, the pharmaceutical agent will be delivered when the percent of macrophages that cross the CFBB will be in excess of 2%, more preferably in excess of 3%, more preferably in excess of 4%, and most preferably in excess of 5%.

Certain viruses and bacteria can be taken up by phagocytic cells and continue to remain within these cells. However, cells loaded with the drug particles are effective in treating such infections because the drug is concentrated in the phagocytic cells, and the infecting organism is exposed to much larger amounts of the drug thereby killing the organism. Furthermore, after passing into the brain, acid-solubilizable particles dissolve due to lower pH levels within the phagocytic cells thereby releasing concentrations of the drug. A concentration gradient is formed with higher concentrations of the pharmaceutical composition within an endosomal body of the phagocytic cells and lesser concentrations outside the endosome. Thus, the contents of the particles within the macrophage are released into the brain for ameliorative purposes. Over time, free viral and bacterial organisms residing in the brain are exposed to the drug at concentrations higher than what is typically able to be delivered through oral administration.

In another preferred embodiment, the pharmaceutical composition as particles is administered directly into the vascular system of a mammalian subject. The particles can be engulfed by phagocytic cells residing in the vascular system or adsorbed onto the cell wall. Once the particle is taken up by the loaded cell, a certain percentage of the loaded cells will

be transported across the blood-brain barrier into the brain in a manner similar to transport across the cerebrospinal fluid-brain barrier.

In another preferred embodiment, the method involves treating a patient having a central nervous system infected with HIV by delivering an anti-HIV composition to the brain using one of the processes described above. Suitable anti-HIV compositions include protease inhibitors. Examples of protease inhibitors include indinavir, ritonavir, saquinavir, and nelfinavir. The anti-HIV composition can also be a nucleoside reverse transcriptase inhibitor. Examples of nucleoside reverse transcriptase inhibitors include zidovudine, didanosine, dtavudine, zalcitabine, and lamivudine. The anti-HIV composition can also be a non-nucleoside reverse transcriptase inhibitor. Examples of non-nucleoside reverse transcriptase inhibitors include nevirapine and delaviradine.

Treatment of HIV infection by nanosuspensions of anti-retroviral agents for increased Central Nervous System (CNS) delivery

HIV-1 associated dementia remains a continuing medical problem, despite the advent of highly active anti-retroviral therapy (HAART). Poor CNS penetration of many anti-retroviral drugs affords only sub-therapeutic drug levels, resulting in development of resistant viral strains. These persist in infecting the brain as well as escape their sanctuaries to infect the systemic circulation. Clearly, superior drug delivery systems are needed for enhanced brain delivery (see Reference 1, Limoges *et al.*).

Monocyte-derived macrophages (MDM) are preferred as a vector for drug delivery of anti-retroviral medication because they are the natural target cell for viral infection of the brain (see Reference 2, Nottet *et al.*), and because they are phagocytic toward drug particulate suspensions (see Reference 3, Moghimi *et al.*). Hence, drug uptake and subsequent delivery to the brain may be expected. The protease inhibitor, indinavir, is preferred as a drug that would remain in particulate form at neutral pH for macrophage uptake, but which would dissolve under the acidic conditions of the phagolysozome, rendering the desired therapeutic efficacy.

Example 1: Indinavir nanosuspensions for increased CNS delivery through macrophage targeting

A nanosuspension formulation of Indinavir (IND) (Composition 1) suitable for macrophage targeting was prepared and demonstrated good physical stability upon storage.

Single dose loading of IND nanosuspension effectively suppressed HIV-1 replication and abrogated virus-associated cytopathicity without affecting measures of cell viability.

IND nanosuspension was prepared by high-pressure homogenization of an aqueous suspension at alkaline pH in the presence of appropriate stabilizing surfactants (see Composition 1). Lipoid E80 is a phospholipid mixture manufactured by Lipoid GmbH. The process was optimized for various parameters including temperature and homogenization cycles. Particle size was measured using light scattering and stability of the suspension was assessed using specifically designed stress tests and short-term stability studies.

**Composition 1**

| Ingredient | Concentration (% w/v) |
|---|---|
| Indinavir | 0.6 |
| Lipoid E80 | 1.2 |
| phosphate buffer | 0.14 |
| sodium chloride | 0.9 |
| pH 8 | |

To assess IND nanosuspension activity, MDM were infected with HIV-1 and virus was removed after 12 hours of exposure. Infected cells were treated overnight with 500 uM drug nanosuspension. Replicate MDM were left untreated as controls (CON). Culture supernatants were collected and assessed for reverse transcriptase (RT) activity every 2 days. MDM viability was determined at 9 days after infection by the thiazolyl blue (MTT) conversion assay.

The volume-weighted mean size of the particles was approximately 1.6 microns, with 99% of the particles (by volume) less than 8.4 microns. Process optimization studies indicated that longer homogenization times and lower temperatures produced smaller particles. The suspension was exposed to multiple stress tests to estimate its long-term stability. As can be seen in FIG. 1, the suspension passed all stress tests. Furthermore, as seen in FIG. 2, the suspension was stable for at least 6 months at 5°C as determined from particle size analysis.

CON HIV-1-infected MDM showed promiment cytopathicity (ballooning, multinucleated giant cells, and cell death) with sustained high levels of RT activity throughout the 9 day observation period. IND nanosuspension MDM showed a 99% decrease in RT activity compared to controls with no cytopathicity. The drug nanosuspension had no statistically significant effects on MDM viability.

While specific embodiments have been illustrated and described, numerous modifications come to mind without departing from the spirit of the invention and the scope of protection is only limited by the scope of the accompanying claims.

**References**(1) J. Limoges, I. Kadiu, D. Morin, M. Chaubal, J. Werling, B. Rabinow, and H.E. Gendelman, "Sustained Antiretroviral Activity of Indinavir Nanosuspensions in Primary Monocyte-Derived Macrophages," poster presentation, 11th Conference on Retroviruses and Opportunistic Infections, Feb. 8-11, 2004, San Francisco.

(2) H.S.L.M. Nottet and S. Dhawan, "HIV-1 entry into Brain: Mechanisms for the infiltration of HIV-1-infected macrophages across the blood-brain barrier" in The Neurology of AIDS, eds H. E. Gendelman, S Lipton, L. Epstein, S. Swindells, 1998, Chapman & Hall, p.55.

(3) S. Moein Moghimi, A. Christy Hunter, and J. Clifford Murray, "Long-Circulating and Target-Specific Nanoparticles: Theory to Practice", Pharmacological Reviews, 53:283-318, 2001

**What is claimed is:**

1. A pharmaceutical composition of an anti-retroviral agent for delivery to a brain of a mammalian subject comprising a dispersion of the pharmaceutical composition provided as particles having an average particle size of from about 100 nm to about 100 microns and adapted for administering to the mammalian subject for delivery to the brain of an effective amount of the pharmaceutical composition by cells capable of reaching the brain.

2. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is administered to a central nervous system of the mammalian subject.

3. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is administered to a vascular system of the mammalian subject.

4. The pharmaceutical composition of claim 3, wherein the pharmaceutical composition is administered to a veinous system of the mammalian subject.

5. The pharmaceutical composition of claim 3, wherein the pharmaceutical composition is administered to a carotid artery of the mammalian subject.

6. The pharmaceutical composition of claim 1, wherein the cells are capable of phagocytosis.

7. The pharmaceutical composition of claim 1, wherein the cells are selected from the group consisting of T-lymphocytes, monocytes, granulocytes, neutrophils, basophils, eosinophils and mixtures thereof.

8. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is taken up as particles by the cells.

9. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is adsorbed as particles on the surface of the cells.

10. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is contacted with the cells as particles.

11. The pharmaceutical composition of claim 10, wherein the pharmaceutical composition is contacted with isolated cells.

12. The pharmaceutical composition of claim 11, wherein the pharmaceutical composition is contacted with cells isolated by a cell separator.

13. The pharmaceutical composition of claim 1, wherein a portion of the particles do not dissolve prior to delivery to the brain.

14. The pharmaceutical composition of claim 1, wherein the dispersion has a concentration of particles above a thermodynamic or apparent solubility of the particles.

15. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition further comprises a surfactant.

16. The pharmaceutical composition of claim 15, wherein the surfactant is selected from the group consisting of anionic surfactants, cationic surfactants, nonionic surfactants and surface active biological modifiers.

17. The pharmaceutical composition of claim 16, wherein the anionic surfactant is selected from the group consisting of: alkyl sulfonates, alkyl phosphates, alkyl phosphonates, potassium laurate, triethanolamine stearate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl inosine, phosphatidylserine, phosphatidic acid and their salts, sodium carboxymethylcellulose, bile acids and their salts, cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, and glycodeoxycholic acid.

18. The pharmaceutical composition of claim 15, wherein the cationic surfactant is selected from the group consisting of: quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium bromide, chitosans, lauryldimethylbenzylammonium chloride, acyl carnitine hydrochlorides and alky pyridinium halides.

19. The pharmaceutical composition of claim 15, wherein the nonionic surfactant is selected from the group consisting of: polyoxyethylene fatty alcohol ethers, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene fatty acid esters, sorbitan esters, glycerol monostearate, polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl

alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers, poloxamines, methylcellulose, hydroxymethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, noncrystalline cellulose, polysaccharides, starch, starch derivatives, hydroxyethylstarch, polyvinyl alcohol, glyceryl esters and polyvinylpyrrolidone.

20. The pharmaceutical composition of claim 19, wherein the polyoxyethylene fatty acid ester is polyethylene-660-hydroxystearate.

21. The pharmaceutical composition of claim 15, wherein the surface active biological modifiers are selected from the group consisting of: albumin, casein, hirudin, or other proteins.

22. The pharmaceutical composition of claim 15, wherein the surface active biological modifiers are polysaccharides.

23. The pharmaceutical composition of claim 22, wherein the polysaccharide is selected from the group consisting of starch, heparin, chitosan and mixtures thereof.

24. The pharmaceutical composition of claim 15, wherein the surfactant comprises a phospholipid.

25. The pharmaceutical composition of claim 24, wherein the phospholipid is selected from natural phospholipids and synthetic phospholipids.

26. The pharmaceutical composition of claim 24, wherein the phospholipid is selected from the group consisting of: phosphatidylcholine, phosphatidylethanolamine, diacyl-glycero-phosphoethanolamine, dimyristoyl-glycero-phosphoethanolamine (DMPE), dipalmitoyl-glycero-phosphoethanolamine (DPPE), distearoyl-glycero-phosphoethanolamine (DSPE), dioleolyl-glycero-phosphoethanolamine (DOPE), phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, lysophospholipids, polyethylene glycol-phospholipid conjugates, egg phospholipid and soybean phospholipid.

27. The pharmaceutical composition of claim 24, wherein the phospholipid further comprises a functional group to covalently link to a ligand.

28. The pharmaceutical composition of claim 27, wherein the ligand is selected from the group consisting of PEGs, proteins, peptides, carbohydrates, glycoproteins, antibodies and pharmaceutically active agents.

29. The pharmaceutical composition of claim 15, wherein the surfactant comprises a bile acid or a salt thereof.

30. The pharmaceutical composition of claim 29, wherein the surfactant is selected from deoxycholic acid, glycocholic acid, glycodeoxycholic acid, taurocholic acid and salts of these acids.

31. The pharmaceutical composition of claim 15, wherein the surfactant comprises a copolymer of oxyethylene and oxypropylene.

32. The pharmaceutical composition of claim 31, wherein the copolymer of oxyethylene and oxypropylene is a block copolymer.

33. The pharmaceutical composition of claim 1, wherein the particles in the dispersion are amorphous, semicrystalline, crystalline, or a combination thereof as determined by XRD.

34. The pharmaceutical composition of claim 1, wherein the anti-retroviral agent is a protease inhibitor.

35. The pharmaceutical composition of claim 34, wherein the protease inhibitor is selected from the group consisting of: indinavir, ritonavir, saquinavir, and nelfinavir.

36. The pharmaceutical composition of claim 1, wherein the anti-retroviral agent is indinavir.

37. The pharmaceutical composition of claim 1, wherein the therapeutic agent is a nucleoside reverse transcriptase inhibitor.

38. The pharmaceutical composition of claim 37, wherein the nucleoside reverse transcriptase inhibitor is selected from the group consisting of: zidovudine, didanosine, stavudine, zalcitabine, and lamivudine.

39. The pharmaceutical composition of claim 1, wherein the therapeutic agent is a non-nucleoside reverse transcriptase inhibitor.

40. The pharmaceutical composition of claim 30, wherein the non-nucleoside reverse transcriptase inhibitor is selected from the group consisting of nevirapine and delaviradine.

41. The pharmaceutical composition of claim 1, wherein the therapeutic agent is used to treat HIV infection in the central nervous system.

42. The pharmaceutical composition of claim 1, wherein the step of providing a dispersion comprises the step of homogenizing the pharmaceutical composition through a homogenization process.

43. The pharmaceutical composition of claim 1, wherein the step of providing a dispersion comprises the step of homogenizing the pharmaceutical composition through a microprecipitation/homogenization process.

44. The pharmaceutical composition of claim 1, wherein the dispersion of the pharmaceutical composition is administered intrathecally or epidurally.

45. The pharmaceutical composition of claim 1, wherein the dispersion of the pharmaceutical composition is sterilized prior to administering.

46. The pharmaceutical composition of claim 45, wherein sterilizing is performed by heat sterilization or gamma irradiation.