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(54) Title: PROCESSES TO GENERATE SUBMICRON PARTICLES OF WATER-INSOLUBLE COMPOUNDS

(57) Abstract

Submicron particles of water-insoluble compounds, particularly drugs, are prepared by simultaneously stabilizing microparticulate suspensions of same with surface modifier molecules by rapid expansion into an aqueous medium from a compressed solution of the compound and surface modifiers in a liquefied gas and optionally homogenizing the aqueous suspension thus formed with a high pressure homogenizer.

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PROCESSES TO GENERATE SUBMICRON PARTICLES OF WATER-INSOLUBLE COMPOUNDS

This invention provides processes for producing micrometer and sub-micrometer sized particulate preparations of biologically useful compounds that are water-insoluble or poorly water-soluble, particularly water-insoluble pharmaceutical agents.

BACKGROUND AND SUMMARY OF INVENTION

A major problem in formulating biologically active compounds is their poor solubility or insolubility in water. For instance, over one third of the drugs listed in the United States Pharmacopoeia are either water-insoluble or poorly water-soluble. Oral formulations of water-insoluble drugs or compounds with biological uses frequently show poor and erratic bioavailability. In addition, drug insolubility is one of the most recalcitrant problems facing medicinal chemists and pharmaceutical scientists developing new drugs. Water-insolubility problems delay or completely block the development of many new drugs and other biologically useful compounds, or prevent the much-needed reformulation of certain currently marketed drugs. Although the water-insoluble compounds may be formulated by solubilization in organic solvents or aqueous-surfactant solutions, in many cases such solubilization may not be a preferred method of delivery of the water-insoluble agent for their intended biological use. For instance, many currently available injectable formulations of water-insoluble drugs carry important adverse warnings on their labels that originate from detergents and other agents used for their solubilization.

An alternative approach for the formulation of water-insoluble biologically active compounds is surface-stabilized particulate preparations. Small particle size formulation of drugs are often needed in order to maximize surface area, bioavailability and, dissolution requirements. Pace et al. ("Novel Injectable Formulations of Insoluble Drugs" in Pharmaceutical Technology, March 1999) have

reviewed the usefulness of the microparticulate preparations of water-insoluble or poorly soluble injectable drugs.

In US patents 5,091,187 and 5,091,188 to Haynes describe the use of phospholipids as surface stabilizers to produce aqueous suspension of submicron sized particles of the water-insoluble drugs. These suspensions are believed to be the first applications of the surface modified microparticulate aqueous suspension containing particles made up of a core of pure drug substances and stabilized with natural or synthetic bipolar lipids including phospholipids and cholesterol. Subsequently, similar delivery systems exploiting these principles have been described (G.G. Liversidge et al., US Patent 5,145,684; K. J. Illig et al. US Patent 5,340,564 and H. William Bosch et al., US Patent 5,510,118) emphasizing the usefulness of the drug delivery approach utilizing particulate aqueous suspensions.

In US patent 5,246,707 Haynes teaches uses of phospholipid-coated microcrystals in the delivery of water-soluble biomolecules such as polypeptides and proteins. The proteins are rendered insoluble by complexation and the resulting material forms the solid core of the phospholipid-coated particle.

These patents and others utilized processes based on the particle size reduction by mechanical means such as attrition, cavitation, high-shear, impaction, etc achieved by media milling, high pressure homogenization, ultrasonication, and microfluidization of aqueous suspensions. However, these particle size reduction methods suffer from certain disadvantage, such as long process duration (high-pressure homogenization or microfluidization) and contamination (media milling, and ultrasonication). In addition, these methods may not be suitable for aqueous suspensions of compounds with limited stability in aqueous medium at the pH, high temperature and high pressure conditions prevailing in these processes.

Among the alternatives that address to these problems is a procedure which uses liquefied gasses for the production of microparticulate preparations. In one such method liquefied-gas solutions are sprayed to form aerosols from which fine solid

particles precipitate. The phenomenon of solids precipitated from supercritical fluids was observed and documented as early as 1879 by Hannay, J. B. and Hogarth, J., "On the Solubility of Solids in Gases," Proc. Roy. Soc. London 1879 A29, 324,

The first comprehensive study of rapid expansion from a liquefied-gas solution in the supercritical region was reported by Krukonis (1984) who formed micro-particles of an array of organic, inorganic, and biological materials. Most particle sizes reported for organic materials, such as lovastatin, polyhydroxyacids, and mevinolin, were in the 5-100 micron range. Nanoparticles of beta-carotene (300 nm) were formed by expansion of ethane into a viscous gelatin solution in order to inhibit post expansion particle aggregation. Mohamed, R. S., et al. (1988), "Solids Formation After the Expansion of Supercritical Mixtures," in Supercritical Fluid Science and Technology, Johnston, K. P. and Penninger, J. M. L., eds., describes the solution of the solids naphthalene and lovastatin in supercritical carbon dioxide and sudden reduction of pressure to achieve fine particles of the solute. The sudden reduction in pressure reduces the solvent power of the supercritical fluid, causing precipitation of the solute as fine particles.

Tom, J. W. and Debenedetti, P. B. (1991), "Particle Formation with Supercritical Fluids--a Review," J. Aerosol. Sci. 22:555-584, discusses rapid expansion of supercritical solutions techniques and their applications to inorganic, organic, pharmaceutical and polymeric materials. This technique is useful to comminute shock-sensitive solids, to produce intimate mixtures of amorphous materials, to form polymeric microspheres and deposit thin films.

Most studies of rapid expansion from supercritical solution on organic materials utilize supercritical carbon dioxide. However, ethane was preferred to carbon dioxide for beta-carotene because of certain chemical interactions. Carbon dioxide is generally preferred, alone or in combination with a cosolvent. Minute additions of a cosolvent can significantly influence the solvent properties. When cosolvents are used in rapid expansion from a supercritical solution, care is required to prevent de-solution of the particles due to solvent condensing in the nozzle. Normally, this is

achieved by heating the supercritical fluid, prior to expansion, to a point where no condensate (mist) is visible at the nozzle tip.

A similar problem occurs when carbon dioxide is used. During adiabatic expansion (cooling), carbon dioxide will be in two phases unless sufficient heat is provided at the nozzle to maintain a gaseous state. Most investigators recognize this phenomenon and increase the pre-expansion temperature to prevent condensation and freezing in the nozzle. A significant heat input is required (40-50 kcal/kg) to maintain carbon dioxide in the gaseous state. If this energy is supplied by increasing the pre-expansion temperature the density drops and consequently reduces the supercritical fluid's solvating power. This can lead to premature precipitation and clogging of the nozzle.

The solvent properties of liquefied-gas are strongly affected by their fluid density in the vicinity of the fluid's critical point. In rapid expansion from liquefied-gas solutions, a non-volatile solute is dissolved in a liquefied-gas that remains either in the supercritical or sub-critical phase. Nucleation and crystallization are triggered by reducing the solution density through rapid expansion of the liquefied-gas to atmospheric conditions. To achieve this the liquefied-gas is typically sprayed through 10-50 micron (internal diameter) nozzles with aspect ratios (L/D) of 5-100. High levels of supersaturation result in rapid nucleation rates and limited crystal growth. The combination of a rapidly propagating mechanical perturbation and high supersaturation is a distinguishing feature of rapid expansion from a liquefied-gas solution. These conditions lead to the formation of very small particles with a narrow particle size distribution.

There are a number of advantages in utilizing compressed carbon dioxide in the liquid and supercritical fluid states, as a solvent or anti-solvent for the formation of materials with submicron particle features. Diffusion coefficients of organic solvents in supercritical fluid carbon dioxide are typically 1-2 orders of magnitude higher than in conventional liquid solvents. Furthermore, carbon dioxide is a small linear molecule that diffuses more rapidly in liquids than do other antisolvents. In the antisolvent

precipitation process, the accelerated mass transfer in both directions can facilitate very rapid phase separation and hence the production of materials with sub-micron features. It is easy to recycle the supercritical fluid solvent at the end of the process by simply reducing pressure. Since supercritical fluids do not have a surface tension, they can be removed without collapse of structure due to capillary forces. Solvent removal from the product is unusually rapid. No carbon dioxide residue is left in the product, and carbon dioxide has a number of other desirable characteristics, for example it is non-toxic, nonflammable, and inexpensive. Furthermore, solvent waste is greatly reduced since a typical ratio of antisolvent to solvent is 30:1.

Exploiting these concepts Henriksen et al. in WO 97/14407, disclosed a process using compressed fluids to produce sub-micron sized particles of water insoluble compounds with biological uses, particularly water insoluble drugs by precipitating a compound by rapid expansion from a supercritical solution in which the compound is dissolved, or precipitating a compound by spraying a solution, in which the compound is soluble, into compressed gas, liquid or supercritical fluid which is miscible with the solution but is antisolvent for the compound. In this manner precipitation with a compressed fluid antisolvent (compressed fluid antisolvent) is achieved.

An essential element of this process is the use of phospholipids and other surface modifiers to alter the surface of the drug particles to prevent particle aggregation and thereby improve both their storage stability and pharmacokinetic properties. This process combines or integrates phospholipids or other suitable surface modifiers such as surfactants, as the aqueous solution or dispersion in which the supercritical solution is sprayed. The surfactant is chosen to be active at the compound-water interface, but is not chosen to be active at the carbon dioxide-organic solvent or carbon dioxide-compound interface when carbon dioxide is used as the supercritical solution. The use of surface modifying agents in the aqueous medium allowed making submicron particles by the compressed fluid antisolvent process without particle aggregation or flocculation.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the size distribution (relative volume v. particle size in nm) of cyclosporine produced in Example 3, and

Figure 2 is a graph showing the size distribution (relative volume v. particle size in nm) of cyclosporine produced in Example 4, and

BRIEF DESCRIPTION OF THE INVENTION

However, this prior process suffered from a very long duration of spray of the supercritical solution to obtain a substantial quantity of the desired product. The long duration of spray-process may be attributed to a slow rate of association of the surface modifier molecules or their assemblies in the aqueous medium with the newly precipitated solute particles.

During experimentation with the process of WO 97/14407 described above it was surprisingly found that incorporation of a surface modifier in both the supercritical (or sub-critical) liquefied gas along with incorporation of a surface modifier in the water insoluble substance allowed one to achieve a very rapid production of surface stabilized nanometer- to micrometer-sized particulate suspensions. The principle feature of the present invention is believed to be rapid attainment of intimate contact of the dissolved drug and the surface modifier during the very fast precipitation step of the drug from their solution in the liquefied gas.

While very rapid precipitation is a characteristic of precipitation of solutes from liquefied gases, the rapid intimate contact with the surface modifier is achieved by having the surface modifiers dissolved in the liquefied-gas containing the dissolved drug. A rapid intimate contact between the surface modifier and the newly formed particle substantially inhibits the crystal growth of the newly formed particle. In addition, if the surface modifier(s) is not included with the dissolved drug the rate at which the liquefied-gas droplet containing the drug is brought into contact with the anti-solvent is much slower if very small stable particles are to be obtained. Thus a key feature of the invention is the high productivity of the process.

Although at least one (first) surface modifier should be dissolved along with the water insoluble substance to be reduced in size in the liquefied gas in the inventive process, additional (second) surface modifying agents of the same or different chemical nature may also be included in the aqueous medium. Further, during or after precipitation the fluid streams may be subjected to additional high shear forces, cavitation or turbulence by a high-pressure homogenizer to facilitate intimate contact of the particle surface and the surface modifier. Thus, in those cases where all the surface modifier is dispersed in the aqueous medium and the liquefied gas contains only the water insoluble substance, additional high shear forces, cavitation or turbulence by a high-pressure homogenizer can be exploited to facilitate the intimate contact of the particle surface and the surface modifier.

Thus, the overall objective of the present invention is to develop a process with high productivity based on the use of liquefied gas solvents, including supercritical fluid technology, that yields surface modifier stabilized suspensions of water insoluble drugs with an average particle size of 50 nm to about 2000 nm and a narrow size distribution. The process is robust, scalable and applicable to a wide range of water-insoluble compounds with biological uses.

DETAILED DESCRIPTION OF THE INVENTION

The present invention includes procedures for using super critical or compressed fluids to form surface modified particles of up to about 2000 nm in size and usually below 1000 nm, desirably less than 500 nm, preferably less than about 200 nm and often in a range of 5 to 100 nm in size. The size of the particles refers to volume weighted mean diameters of these particles suspended in aqueous medium.

The process of the present invention includes forming aqueous microparticulate suspensions of water insoluble or poorly water soluble compounds while simultaneously stabilizing of same with surface modifier molecules by rapid expansion into an aqueous medium from a compressed solution of the compound and

surface modifiers in a liquefied-gas (Rapid Expansion of Liquefied Gas Solution, RELGS).

Alternatively another embodiment of the invention includes forming aqueous microparticulate suspensions of water insoluble or poorly water soluble compounds while simultaneously stabilizing the same with surface modifier molecules by rapid expansion into an aqueous medium of a compressed solution of the compound and surface modifiers in a liquefied-gas and homogenizing the aqueous suspension thus formed with a high pressure homogenizer (Rapid Expansion of Liquefied-Gas Solution and Homogenization, RELGS-H).

While not wishing to be bound by any particular theory, the processes of this invention are believed to induce rapid nucleation of the liquefied-gas dissolved drugs and other biologically active substances in the presence of surface modifying agents resulting in particle formation with a desirable size distribution in a very short time. Phospholipids or other suitable surface modifiers such as surfactants, as may be required, may be integrated into the processes as a solution or dispersion in the liquefied gas. In addition, the surface modifier may or may not be incorporated via its solution or dispersion in the aqueous medium. Alternatively, some of the surface modifiers may be dissolved in the liquefied gas along with the water insoluble substance and expanded into a homogenized aqueous dispersion of rest of the surface modifier of the formulation. The introduction of suitable surface modifying agents in the above noted processes serves to stabilize the generated small particles and suppress any tendency of particle agglomeration or particle growth while they are formed.

By industrially useful insoluble or poorly soluble compounds we include biologically useful compounds, imaging agents, pharmaceutically useful compounds and in particular drugs for human and veterinary medicine. Usually, the water insoluble compounds are those having a poor solubility in water, that is less than 5 mg/mL at a physiological pH of 6.5 to 7.4, although the water solubility may be less than 1 mg/mL and even less than 0.1 mg/mL.

Examples of some preferred water-insoluble drugs include immunosuppressive and immunoactive agents, antiviral and antifungal agents, antineoplastic agents, analgesic and anti-inflammatory agents, antibiotics, anti-epileptics, anesthetics, hypnotics, sedatives, antipsychotic agents, neuroleptic agents, antidepressants, anxiolytics, anticonvulsant agents, antagonists, neuron blocking agents, anticholinergic and cholinomimetic agents, antimuscarinic and muscarinic agents, antiadrenergic and antiarrhythmics, antihypertensive agents, antineoplastic agents, hormones, and nutrients. A detailed description of these and other suitable drugs may be found in *Remington's Pharmaceutical Sciences, 18th edition, 1990,* Mack Publishing Co. Philadelphia, PA.

A range of compressed gases in the supercritical or sub-critical fluid phases have been reported in the prior art (for example, US patent 5,776,486, and Tom, J. W. and Debenedetti, P. B. (1991), "Particle Formation with Supercritical Fluids--a Review," J. Aerosol. Sci. 22:555-584) from which a suitable gas may be selected for the purpose of the present invention. These include but are not limited to gaseous oxides such as carbon dioxide and nitrous oxide; alkanes such as ethane, propane, butane, and pentane; alkenes such as ethylene and propylene; alcohols such as ethanol and isopropanol; ketones such as acetone; ethers such as dimethyl or diethyl ether; esters such as ethyl acetate; halogenated compounds including sulfur hexafluoride, chlorofluorocarbons such as trichlorofluoromethane (CCl_3F, also known as Freon 11), dichlorofluoromethane ($CHCl_2F$, also known as Freon 21), difluorochloromethane ($CHClF_2$, also known as Freon 22), and fluorocarbons such as trifluoromethane (CHF_3, also known as Freon 23); and elemental liquefied gases such as xenon and nitrogen and other liquefied compressed gases known to the art.

Liquefied carbon dioxide was used to prepare rapid expansion solutions of the drugs described in the following examples. Carbon dioxide has a critical temperature of 31.3 degrees C. and a critical pressure of 72.9 atmospheres (1072 psi), low chemical reactivity, physiological safety, and relatively low cost. Another preferred supercritical fluid is propane.

Examples of some suitable surface modifiers include: (a) natural surfactants such as casein, gelatin, natural phospholipids, tragacanth, waxes, enteric resins, paraffin, acacia, gelatin, and cholesterol, (b) nonionic surfactants such as polyoxyethylene fatty alcohol ethers, sorbitan fatty acid esters, polyoxyethylene fatty acid esters, sorbitan esters, glycerol monostearate, polyethylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, poloxamers, polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, and synthetic phospholipids, (c) anionic surfactants such as potassium laurate, triethanolamine stearate, sodium lauryl sulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, negatively charged phospholipids (phosphatidyl glycerol, phosphatidyl inosite, phosphatidylserine, phosphatidic acid and their salts), and negatively charged glyceryl esters, sodium carboxymethylcellulose, and calcium carboxymethylcellulose, (d) cationic surfactants such as quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium bromide, and lauryldimethylbenzyl-ammonium chloride, (e) colloidal clays such as bentonite and veegum, (f) natural or synthetic phospholipid, for example phosphatidylcholine, phosphatidylethanolamine, 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. A detailed description of these surfactants may be found in Remington's Pharmaceutical Sciences, 18th Edition, 1990, Mack Publishing Co., PA; and Theory and Practice of Industrial Pharmacy, Lachman et al., 1986.

The following examples further explain and illustrate the invention:

Example 1:

Phase Behavior of Water Insoluble Compound in Compressed Liquefied Gasses.

In order to assess whether a particular water insoluble compound should be formulated as an aqueous submicron particulate suspension from its solution in the

liquefied gasses, the solubility of the candidate drugs in the liquefied gasses was measured.

To prepare solutions with a constant molar composition, measured amounts of drug (fenofibrate) were charged to a constant volume view cell. Temperature was kept constant at 60°C. Pressure was varied from 1300 to 4000 psi by pumping the compressed liquefied gas into the view cell. The phase behavior was determined visually by noting the pressure at which the solid drug appeared to dissolve. A summary of fenofibrate solubility in liquefied carbon dioxide, propane and ethane is given in Table I. The solubility values of >1% w/w in any solvent would allow the fine-particulate preparation from these solvents.

Table I: Fenofibrate Solubility Experiment in Liquefied Carbon Dioxide, Propane and Ethane at 60°C.

Liquefied gas	Pressure (psi)	Solubility (%, w/w)
Carbon Dioxide	1800	0.01
	2000	0.08
	2800	1.4
Propane	1500	2.5
	2000	2.3
Ethane	1300	0.016
	2000	0.79
	3000	1.80
	4000	1.90

Example 2

Fenofibrate Microparticle Formation by the RELGS Process

A solution containing Fenofibrate (2g), Lipoid E-80 (0.2g), Tween-80 (0.2g) in the liquefied carbon dioxide pressurized to 3000 psi was expanded through a 50 mm orifice plate into water held at atmospheric pressure and room temperature (22°C). A fine suspension of fenofibrate was obtained with a mean particle size of about 200 nm. The particle sizing was performed by photon correlation spectroscopy using Submicron Particle Sizer-Autodilute Model 370 (NICOMP Particle Sizing Systems, Santa Barbara, CA). This instrument provides number weighted, intensity weighted,

and volume weighted particle size distributions as well as multi-modality of the particle size distribution, if present.

Example 3

A fine spray-nozzle was constructed with PEEK capillary tubing of an internal diameter of 63.5 mm. This PEEK nozzle was fastened with a M-100 Minitight male nut and attached to an Upchurch SS20V union body which was further attached to a ¼ inch high pressure manifold via Swagelok™ brand fittings of appropriate size. Except for the PEEK tubing all other components were made up of 316 stainless steel. A liquefied gas solution of the water insoluble substance was introduced at high pressure (>1000 psig) through the ¼ inch high pressure manifold into the 63.5mm PEEK nozzle to be expanded into the aqueous medium. The vessel for the liquefied gas solution was charged with 1 g of cyclosporine and 0.2 g of Tween-80. The vessel was filled with carbon dioxide at 5000 psig and heated to about 24°C. The vessel was allowed to stand for about 20 minutes for complete dissolution and for attaining equilibrium. Separately, a 2% w/w suspension of egg phospholipid (Lipoid E80 from Lipoid GmbH) in a 5.5% solution of mannitol was homogenized at 6000 psi with an Avestin Emulsiflex C50 homogenizer (Avestin Inc, Ottawa, Canada) for 15 min when it produced a clear dispersion. The pH of the phospholipid suspension was adjusted to 8.0 with aqueous NaOH solution prior to homogenization. The carbon dioxide solution of cyclosporine and Tween-80 that was held at 24°C and 5000 psig was expanded into the aqueous dispersion of egg phospholipid. Very rapidly, in about 3 minutes, a translucent aqueous suspension of about 23 nanometer particle size was obtained (See Figure 1). This example provides a simple scalable process by the way of incorporation of several such PEEK nozzles within a manifold and simultaneously expanding into a reservoir containing appropriate amount of the aqueous medium. The PEEK nozzle is known to be inert and very inexpensive. Construction of the nozzle is very simple and can be done in less than 10 minutes.

Example 4

Cyclosporine Microparticle Formation by the RELGS-H Process

An aqueous suspension containing Mannitol (5.5%), Lipoid E-80 (2%), and Tween 80 (2%), was prepared. A solution of cyclosporine in the Liquifed gas was also prepared and kept at 2000 psig and 60°C. This solution was expanded through a 63.5 mm PEEK nozzle into the aqueous suspension. A suspension of about 3 g cyclosporine was made in this way. The resulting suspension was homogenized for 8 passes at 6,000 psig. The final mean particle size after homogenization was 86 nanometers with the 99 percentile at 150 nm (see Figure 2) as measured using the Submicron Particle Sizer-Autodilute Model 370 (NICOMP Particle Sizing Systems, Santa Barbara, CA).

While the invention has been described in connection with what is presently considered to be the most practical and preferred embodiment, it is to be understood that the invention is not to be limited to the disclosed embodiment, but on the contrary, is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

WHAT IS CLAIMED IS:

1. A process of preparing a suspension of sub-micron particles of a water-insoluble or substantially water-insoluble biologically active compound of up to 2000 nm in size comprising the steps of:

(a) dissolving a water-insoluble or substantially water-insoluble biologically active compound and a first surface modifier in a liquefied compressed gas solvent therefor and forming a solution; and

(b) expanding the compressed fluid solution prepared in step (a) into water or aqueous solution containing a second surface modifier and water-soluble agents thereby producing a suspension of microparticles..

2. The process of claim 1 including the additional step of
(c) high pressure homogenizing the suspension resulting from step (b).

3. The process according to claim 1 or 2, including the additional step of
(d) recovering the microparticles so produced.

4. The process according to claim 1 or 2, wherein the first surface modifier and the second surface modifier are the same.

5. The process according to claim 1 or 2, wherein the first surface modifier and the second surface modifier are different.

6. The process according to claim 1 or 2, wherein one or both of the surface modifiers is a phospholipid.

7. The process according to claim 1 or 2, wherein one or both the surface modifiers is a surfactant.

8. The process of claim 1 or claim 2 wherein one or both of the surface modifiers is a mixture of two or more surfactants.

9. The process according to claim 1 or 2, wherein at least one surface modifier is a surfactant devoid or substantially completely devoid of phospholipid.

10. The process of claim 7 wherein the surface modifier is a polyoxyethylene sorbitan fatty acid ester, a block copolymer of ethylene oxide and propylene oxide, a tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylenediamine, an alkyl aryl polyether sulfonate, polyethylene glycol, hydroxy propylmethylcellulose, sodium dodecylsulfate, sodium deoxycholate, cetyltrimethylammonium bromide or combinations thereof.

11. The process of claim 6 wherein the surface modifier is of egg or plant phospholipid or semisynthetic or synthetic in partly or fully hydrogenated or in a desalted or salt phospholipid such as phosphatidylcholine, phospholipon 90H or dimyristoyl phosphatidylglyerol sodium salt, phosphatidylethanolamine, phosphatidylserine, phosphatidic acid, lysophospholipids or combinations thereof.

12. The process of claim 1 or 2 wherein the compound is a cyclosporine, fenofibrate, or alphaxalone.

13. The process of claim 1 or 2 wherein the particles produced are less than 500 nm in size

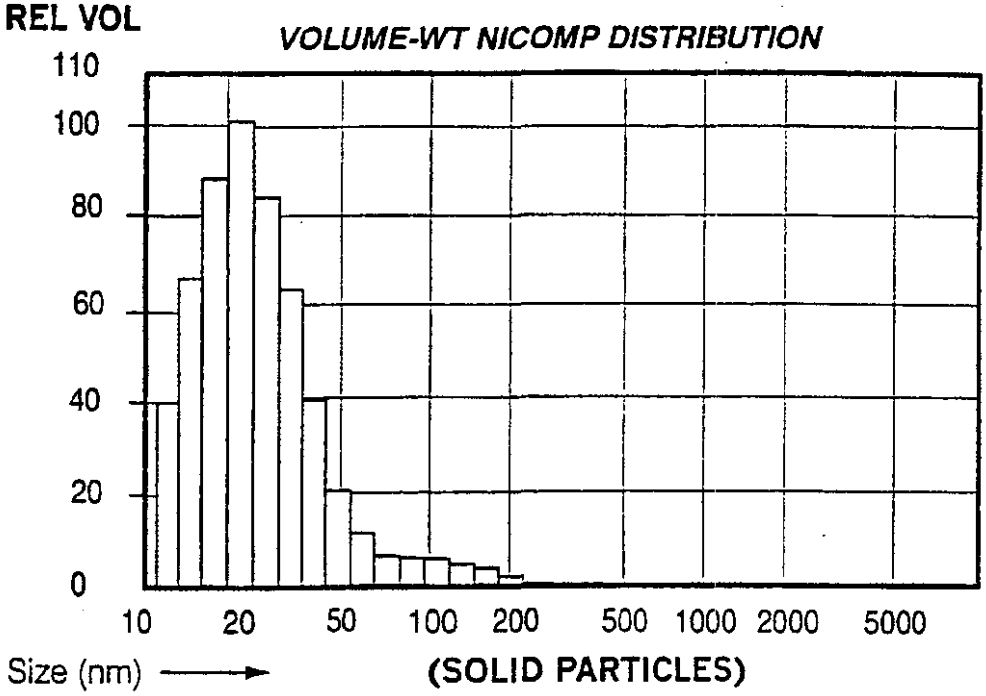
14. The process of claim 13 wherein the particles produced range from 5 up to about 200 nm in size.

15. The process of claim 1 or 2 wherein 99% of the particles produced are below 2000 nm.

16. The process of claim 1 or 2 wherein the liquefied compressed gas is carbon dioxide in the supercritical or sub-critical phase.

VOLUME-WEIGHTED NICOMP DISTRIBUTION ANALYSIS (SOLID PARTICLES)

NICOMP SUMMARY:
Peak Number 1 : Mean Diameter = 22.7 nm Volume: 100.00 %



Mean Diameter = 30.1 nm Pit error = 3.897 Residual = 2.015

NICOMP SCALE PARAMETERS:
Min. Diam. = 10.0 nm Plot Size = 36
Smoothing = 4 Plot Range = 1000

Run Time	= 0 Hr 7 Min 26 Sec	Wavelength	= 632.8 nm
Count Rate	= 301 Khz	Temperature	= 21 deg C
Channel #1	= 267.8 K	Viscosity	= 0.933 cp
Channel Width	= 11.0 uSec	Index of Ref.	= 1.333

GAUSSIAN SUMMARY:

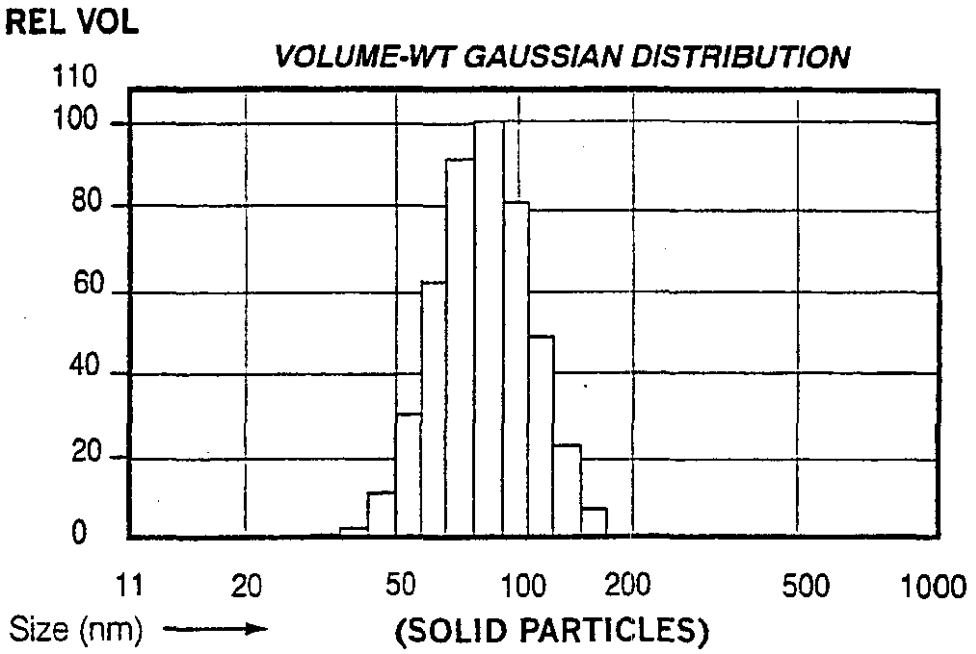
Mean Diameter	= 37.7 nm	Chi Squared	= 36.603
Stnd. Deviation	= 22.1 nm (58.6%)	Baseline Adj.	= 0.016%
Coeff. of Var'n	= 0.586	Mean Diff. Coeff.	= 1.24E-07 cm^2/s

Fig. 1

VOLUME-WEIGHTED GAUSSIAN ANALYSIS (SOLID PARTICLES)

GAUSSIAN SUMMARY:

Mean Diameter	= 86.0 nm	Chi Squared	= 0.218
Stnd. Deviation	= 24.9 nm (29.0%)	Baseline Adj.	= 0.012%
Coeff. of Var'n	= 0.290	Mean Diff. Coeff.	= 5.37E-08 cm^2/s



Cumulative Results:

25 % of distribution < 62.68 nm
50 % of distribution < 76.22 nm
75 % of distribution < 93.05 nm
99 % of distribution < 151.39 nm

Run Time	= 0 Hr 7 Min 12 Sec	Wavelength	= 632.8 nm
Count Rate	= 324 Khz	Temperature	= 21 deg C
Channel #1	= 310.1 K	Viscosity	= 0.933 cp
Channel Width	= 12.0 uSec	Index of Ref.	= 1.333

Fig. 2

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权利要求书2页 说明书11页 附图页数2页

[54]发明名称 生产水不溶性化合物的亚微粒子的方法

[57]摘要

通过使化合物和表面修饰剂的液化气体溶液快速膨胀进入含水介质,同时用表面修饰剂分子稳定所述物质的微粒悬浮体,并任选用高压均化器对由此形成的含水悬浮体进行均化,从而制备水不溶性化合物、尤其药物的亚微颗粒。

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权 利 要 求 书

1. 制备水不溶性或基本不溶于水的、具有生物活性化合物的、粒度高达2000nm的亚微颗粒悬浮体的方法，其包括以下步骤:

(a) 将水不溶性或基本不溶于水的生物活性化合物和第一表面修饰剂溶于液化的压缩气体溶剂并形成溶液；和

(b) 使步骤(a)制备的压缩流体溶液膨胀进入水或含有第二表面修饰剂和水增溶剂的水溶液中，由此生成微粒悬浮体。

2. 权利要求1的方法，其包括另一个步骤

(c) 高压均化由步骤(b)所得的悬浮体.

3. 根据权利要求1或2的方法，其包括另一个步骤:

(d) 回收所生成的微粒。

4. 根据权利要求1或2的方法，其中第一表面修饰剂和第二表面修饰剂是相同的.

5. 根据权利要求1或2的方法，其中第一表面修饰剂和第二表面修饰剂是不同的.

6. 根据权利要求1或2的方法，其中一种或两种表面修饰剂为磷脂。

7. 根据权利要求1或2的方法，其中一种或两种表面修饰剂为表面活性剂.

8. 权利要求1或2的方法，其中一种或两种表面修饰剂为两种或多种表面活性剂的混合物.

9. 根据权利要求1或2的方法，其中至少一种表面修饰剂不含表面活性剂或基本上不含磷脂.

10. 权利要求7的方法，其中表面修饰剂为聚氧乙烯脱水山梨醇脂肪酸酯、环氧乙烷和环氧丙烷的嵌段共聚物、源于顺序添加环氧乙烷和环氧丙烷至乙二胺中所得的四官能嵌段共聚物、烷基芳基聚醚磺酸盐、聚乙二醇、羟丙基甲基纤维素、十二烷基硫酸钠、脱氧胆酸钠、

十六烷基三甲基溴化铵或其组合。

11. 权利要求 6 的方法，其中表面修饰剂为卵磷脂或植物磷脂或半合成或部分合成或完全氢化或脱盐或盐化的磷脂，如卵磷脂、phospholipon 90H 或二肉豆蔻酰磷脂酰甘油钠盐、磷脂酰乙醇胺、磷脂酰丝氨酸、磷脂酸、溶血磷脂或其组合。

12. 权利要求 1 或 2 的方法，其中所述化合物为环孢菌素、非诺贝特或 alphaxalone。

13. 权利要求 1 或 2 的方法，其中所制颗粒的粒度小于 500 纳米。

14. 权利要求 13 的方法，其中所制颗粒的粒度为 5 纳米至约 200 纳米。

15. 权利要求 1 或 2 的方法，其中所制颗粒的 99%小于 2000 纳米。

16. 权利要求 1 或 2 的方法，其中液化压缩气体为超临界或次临界相的二氧化碳。

01.02.14

说　明　书

生产水不溶性化合物的亚微粒子的方法

本发明提供生产具有生物用途化合物的微米和亚微米级颗粒制剂的方法，这些化合物为水不溶性或水溶性差，尤其水不溶性的药剂。

发明背景及概述

配制生物活性化合物的主要问题是它们的水溶性差或不溶于水。例如，在收录于美国药典的药物中，超过三分之一的药物为水不溶性或水溶性差。具有生物用途的水不溶性药物或化合物的口服制剂通常显示出较差和不稳定的生物利用率。另外，药物的不溶性是药剂师和药物科学家在开发新药时所面临的最棘手的问题之一。水不溶性问题延误或彻底阻碍了许多新药或其它生物用途化合物的开发，或阻止对当前市场上流行药物的非常需要的重新配制。尽管一些水不溶性化合物可以通过增溶于有机溶剂或表面活性剂水溶液来进行配制，但在许多情况下，这种增溶可能不是传递用于生物用途的水不溶性试剂的优选方法。例如，目前许多供注射用的水不溶药物制剂在其标签上对它们增溶时所用的洗涤剂和其它试剂注有重要的不利的警告。

配制水不溶的生物活性化合物的另一种方法为表面稳定的颗粒制剂。通常需要将药物配制成小粒度从而增大表面积、生物利用率以及溶出要求。Pace 等("不溶性药物的新的注射制剂", 药学技术, 1999 年 3 月)对水不溶性或溶解性差的注射药物的微粒制剂的应用进行了综述。

Haynes 在美国专利第 5091187 和 5091188 号描述了使用磷脂作为表面稳定剂以生产水不溶性药物的亚微级颗粒的水悬浮体。这些

悬浮体被认为首先用于表面改性的微粒的水悬浮体，其含有由纯药物核组成的颗粒并且用天然或合成的双极性类脂(包括磷脂和胆固醇)进行稳定。因此，已经有人对采用这些要素的传递系统进行了描述(G. G. Liversidge 等，美国专利第 5145684 号；K. J. Illig 等，美国专利第 5340564 号和 H. William Bosch 等，美国专利第 5510118 号)，其重点为采用颗粒的水悬浮体的药物传递方法的用途。

Haynes 在美国专利第 5246707 号中描述了采用磷脂包膜的微晶在水溶性生物分子如多肽和蛋白质的传递中的应用。这些蛋白质通过络合具有不溶性并且所得物质形成磷脂包膜颗粒的固体核。

这些专利和其它文献采用基于由机械方法如磨擦、空化、高剪切、冲击等来减小粒度的方法，这些机械方法通过对水悬浮体的介质研磨、高压均化、超声和微流化(microfluidization)来获得。然而，这些减小粒度的方法具有某些缺陷，如长的处理时间(高压均化或微流化)和污染(介质研磨和超声波处理)。此外，这些方法可能不适用于在这些方法中普遍存在的 pH、高温和高压条件下的水性介质中的具有有限稳定性的化合物的水悬浮体。

解决这些问题的替代方法的一种是采用液化气来生产微粒制剂。在一种这样的方法中，对液化气溶液进行喷雾以形成微细固体颗粒从中沉淀的气雾剂形式。可以观察到从超临界流体沉淀出固体的现象，并且早在 1879 年已由 Hannay, J. B.和 Hogarth, J.在“固体在气体中的溶解性”(Proc. Roy. Soc. London 1879 A29, 324)中进行了叙述。

Krukonis(1984)对在超临界区域中的液化气溶液快速膨胀第一次进行了综合性研究，他对有机、无机和生物材料微粒进行了排列。据报导，有机材料如 lovastatin、多羟基酸以及 mevinolin 的粒度范围大多为 5-100 微米。通过将乙烷膨胀至粘稠的明胶溶液从而抑制聚集颗粒的后膨胀，可以形成β-胡萝卜素的纳米颗粒(300nm)。Mohamed, R. S.等在(1988)“超临界混合物膨胀后形成的固体”(超临界科学和技术，

Johnston, K. P.和 Penninger, J. M. L.编)中对固体萘和 lovastatin 的超临界二氧化碳溶液和突然减小压力以获得所述溶质的微细颗粒进行了描述。压力的突然减小降低了超临界流体的溶剂性能(power)，使溶质以微细颗粒的形式沉淀出来。

Tom, J. W.和 Debenedetti, P. B.(1991)在“用超临界流体形成颗粒-综述”(J. Aerosol. Sci. 22: 555-584)中描述了超临界溶液技术的快速拓展以及它们在无机物、有机物、药物以及聚合物中的应用。这种技术适用于粉碎冲击敏感的固体、生成无定形材料的紧密混合物、形成聚合物微球以及沉积薄膜。

从超临界流体中快速膨胀有机物的大多数研究均采用超临界的二氧化碳。然而，对于β-胡萝卜素而言，由于某些化学作用，乙烷优于二氧化碳。通常优选单独或与共溶剂组合的二氧化碳。微量加入共溶剂可以明显影响溶剂性质。当共溶剂用于超临界溶液的快速膨胀时，要注意防止由于在喷嘴上溶剂冷凝而引起的颗粒的脱溶解。通常通过在膨胀前将超临界流体加热至喷嘴尖端没有观察到冷凝物(水雾)来实现它。

当采用二氧化碳时出现了相似的问题。在绝热膨胀(冷却)时，除非向喷嘴提供足够的热量从而保持气态，否则二氧化碳将分成两相。多数研究者注意到了这种现象并增大预膨胀温度从而防止喷嘴的冷凝和冷冻。需要高的热量输入(40-50 kcal/kg)来保持二氧化碳为气态。如果这种能量通过增大预膨胀温度来提供，那么密度将减小并因此降低超临界流体的溶剂化能力。这将引起过早的沉淀和喷嘴的堵塞。

液化气的溶剂性质在很大程度上受接近流体临界点时它们的流体密度的影响。在液化气溶液的快速膨胀中，不挥发性溶质溶于保持超临界或次临界相的液化气中。通过液化气快速膨胀至大气条件下来降低溶液密度，从而触发成核和结晶。为实现这一点，通常将液化气通过长径比(L/D)5-100、内径 10-50 微米的喷嘴进行喷雾。高水平的过饱和导致快速成核和有限的晶体生长。快速传播的机械搅

动和高度过饱和的结合为液化气溶液快速膨胀的显著特征。这些条件导致形成具有较窄粒度分布的极小颗粒。

在液态和超临界流体状态下，采用压缩二氧化碳作为形成具有亚微颗粒特性材料的溶剂或反溶剂(anti-solvent)时具有许多优点。有机溶剂在超临界二氧化碳流体中的扩散系数通常比在常规液体溶剂中高 1-2 个数量级。另外，二氧化碳为小的线性分子，在液体中的扩散要快于其它的反溶剂。在反溶剂的沉淀过程中，在各个方向上加速的传质可以促进极快的相分离并由此生产具有亚微特性的材料。在本方法的末尾易于通过简单的减压来循环超临界流体溶剂。由于超临界流体没有表面张力，因此可以通过毛细力在不破坏结构的前提下来脱除它们。从产物中脱除溶剂非常快。在产物中没有残余的二氧化碳，并且二氧化碳具有许多其它所需的特性，例如，它无毒、不燃并且便宜。另外，由于反溶剂与溶剂之比通常为 30:1，可大大减少废溶剂。

Henriksen 等在 WO 97/14407 中提出的这些理论，公开了采用压缩流体来生产具有生物用途的水不溶性化合物尤其水不溶性药物的亚微粒度颗粒的方法，它通过从化合物溶于其中的超临界溶液中快速膨胀来沉淀化合物，或者通过将所述化合物溶于其中的溶液喷雾到与所述溶液可以混溶、但为所述化合物的反溶剂的压缩气体、液体或超临界流体中来进行沉淀。可以这种方式用压缩流体反溶剂(压缩流体反溶剂)进行沉淀。

本方法的基本要素是使用磷脂和其它的表面修饰剂改性药物颗粒的表面从而防止颗粒的聚集并因此提高它们的贮存稳定性和药物动力学特性。这种方法结合或组合磷脂或其它合适的表面修饰剂如以水溶液或分散体(其中对超临界溶液进行喷雾)的形式存在的表面活性剂。当使用二氧化碳用作超临界溶液时，选择对化合物-水界面具有活性的表面活性剂，但不能选择对二氧化碳-有机溶剂或二氧化碳-化合物界面具有活性的表面活性剂。在没有颗粒聚集或絮凝的条件

下，可以通过压缩流体反溶剂的方法，在水介质中采用表面修饰剂来制备亚微颗粒。

附图简述

图 1 图示了由实施例 3 生产的环孢菌素的粒度分布(相对体积对粒度 nm)，和

图 2 图示了由实施例 4 生产的环孢菌素的粒度分布(相对体积对粒度 nm)。

发明综述

然而，在这种先有方法中，需要对超临界溶液进行非常长时间的喷雾才能获得大量所需的产品。长时间的喷雾过程可以归因于表面修饰剂分子或它们的聚集体在含有新的沉淀溶质颗粒的水介质中的慢速缔合。

在上面所述 WO 97/14407 方法的实验中，人们惊奇地发现：在超临界(或次临界)液化气中加入表面修饰剂，以及在不溶性物质中加入表面修饰剂可以很快地生产表面稳定的纳米至微米级的颗粒悬浮体。本发明的主要特性被认为是在药物和从其液化气溶液的极快沉淀过程中，快速获得溶解药物和表面修饰剂的紧密接触。

由于快速沉淀是溶质从液化气中沉淀的一个特性，与表面修饰剂的快速紧密接触可以通过将表面修饰剂溶于含有溶解药物的液化气中来获得。表面修饰剂与新形成的颗粒药物之间的快速紧密接触基本抑制了新形成颗粒的晶体生长。另外，如果要获得非常小的稳定颗粒，如果表面修饰剂没有加入溶解药物中，那么含有药物的液化气液滴与反溶剂接触时的速率要慢许多。因此本发明的关键特性是本方法的高生产率。

尽管在本发明方法中应有至少一种(第一)表面修饰剂与需降低尺寸的水不溶性物质一起溶于液化气，但具有相同或不同化学特性的

另一种(第二)表面修饰剂也可包括在水介质中．另外，在沉淀中或其后，可以将所述流体通过高压均化器来施加另外的高剪切力、空化或湍流，从而促进颗粒表面与表面修饰剂的紧密接触．因此，在将所有的表面修饰剂分散至水介质中并且液化气只含有水不溶性物质的情况下，可以通过高压均化器，采用另外的高剪切力、空化或湍流来促进颗粒表面和表面修饰剂的紧密接触．

因此，本发明的总目标是开发包含超临界流体技术的、基于采用液化气溶剂的高生产率方法，这种方法可以得到表面修饰剂稳定的水不溶性药物，其平均粒度为 50nm 至约 2000nm 并且具有窄的粒度分布．本方法是成熟的、规模化方法并且广泛适用于具有生物用途的水不溶性化合物．

发明详述

本发明包括采用超临界流体或压缩流体来形成表面修饰的颗粒，其粒度可高达约 2000nm，通常小于 1000nm，理想小于 500nm，优选小于约 200nm，并且通常为 5-100nm 的范围内．颗粒的粒度是指水介质中悬浮的这些颗粒的容重平均直径．

本发明方法包括形成水不溶性或水溶性差的化合物的含水的微粒悬浮体，同时通过使液化气中的化合物和表面修饰剂从压缩溶液快速膨胀至水性介质中，从而使表面修饰剂分子稳定所述物质(液化气溶液的快速膨胀，RELGS)．

本发明的另一个实施方案包括形成水不溶性或水溶性差的化合物的含水微粒悬浮体，同时通过使液化气中的化合物和表面修饰剂从压缩溶液快速膨胀至水性介质中，从而使表面修饰剂分子稳定所述物质，并用高压均化器均化由此形成的含水悬浮体(液化气溶液的快速膨胀和均化，RELGS-H)．

不希望受到具体理论的束缚，本发明方法被认为在表面修饰剂的存在下，使溶于液化气的药物和其它生物活性物质快速成核，从

而在极短的时间内形成所需粒度分布的颗粒。如果需要，可以将磷脂或其它合适的表面修饰剂(如表面活性剂)以液化气的溶液或分散体的形式加入本方法中。另外表面修饰剂可选择以其水介质的溶液或分散体的形式加入。或者，可以将一些表面修饰剂与水不溶性物质一起溶于液化气中并且膨胀至所述制剂的其余表面修饰剂的均化的含水分散体中。在上述方法中引入的合适的表面修饰剂用来稳定生成的小颗粒并且在形成颗粒时，抑制颗粒的聚集或生长。

我们所指的工业上有用的不溶性或溶解性差的化合物包括具有生物用途的化合物、显影剂、药物，尤其用于人类和动物的药物。通常水不溶性的化合物为那些在水中溶解性差的化合物，即在生理pH6.5-7.4 时溶解性小于 5mg/ml 的化合物，也可能是水溶性小于 1mg/ml，甚至小于 0.1mg/ml 的化合物。

一些优选的水不溶性药物的例子包括抑制免疫和具有免疫活性的药剂，抗病毒剂和抗真菌剂、抗肿瘤药、止痛剂和消炎药、抗生素、镇癫痫药、麻醉剂、催眠剂、镇静剂、精神抑制剂、精神安定药、抗抑郁剂、抗焦虑药、抗惊厥药、拮抗剂、神经元阻滞剂(neuron blocking agents)、抗胆碱能药和类胆碱能药、抗毒蕈碱药和毒蕈碱药、抗肾上腺素能药和抗心律失常药、抗高血压药、抗肿瘤药、荷尔蒙和营养素。这些和其它合适的药物可参见雷明顿药物科学(第 18 版，1990，Mack Publishing Co. Philadelphia, PA)。

在先有技术中对超临界或次临界流体相中的压缩气体进行了广泛的报导(如美国专利第 5776486 号以及 Tom, J. W.和 Debenedetti, P. B.(1991)的“用超临界流体形成颗粒-综述”J. Aerosol. Sci. 22: 555-584)，从中可以选择合适的气体用于本发明目的。这些气体包括但并不局限于气体氧化物如二氧化碳和氧化亚氮；烷烃如乙烷、丙烷、丁烷和戊烷；烯烃如乙烯和丙烯；醇如乙醇和异丙醇；酮如丙酮；醚如二甲醚或二乙醚；酯如乙酸乙酯；卤代化合物包括六氟化硫、含氯氟烃如三氯氟甲烷(CCl_3F，也称氟利昂 11)、二氯氟甲烷($CHCl_2F$，

也称氟利昂 21)、二氯氟甲烷($CHClF_2$，也称氟利昂 22)、以及碳氟化合物如三氟甲烷(CHF_3，也称氟利昂 23)；以及元素液化气体如氙和氮以及本领域熟知的其它液化压缩气体。

在下面的实施例中，液化二氧化碳用于制备药物的快速膨胀溶液。二氧化碳的临界温度为 31.3 度，临界压力为 72.9 个大气压(1072 psi)，化学活性差、生理学上安全并且成本较低。另一种优选的超临界流体为丙烷。

合适的表面修饰剂的实例包括：(a) 天然表面活性剂如酪蛋白、明胶、天然磷脂、黄蓍胶、蜡、包囊树脂(enteric resins)、石蜡、阿拉伯胶、明胶以及胆固醇，(b) 非离子表面活性剂如聚氧乙烯脂肪醇醚、脱水山梨醇脂肪酸酯、聚氧乙烯脂肪酸酯、脱水山梨醇酯、单硬脂酸甘油酯、聚乙二醇、十六醇、十六醇和十八醇的混合物、十八烷醇、poloxamers、polaxamines、甲基纤维素、羟基纤维素、羟丙基纤维素、羟丙基甲基纤维素、非晶体纤维素以及合成磷脂，(c) 阴离子表面活性剂如月桂酸钾、硬脂酸三乙酸醇胺、月桂基硫酸钠、烷基聚氧乙烯基硫酸盐、海藻酸钠、二辛基磺基琥珀酸钠、带负电的磷脂(磷脂酰甘油、磷脂酰肌醇、磷脂酰丝氨酸、磷脂酸和它们的盐)、带负电的甘油酯、羧甲基纤维素钠和羧甲基纤维素钙，(d) 阳离子表面活性剂如季铵化合物、氯化苯甲烃铵、十六烷基三甲基溴化铵和月桂基二甲基苄基氯化铵，(e) 膨润土如皂土和胶体镁铝硅酸盐，(f) 天然或合成的磷脂，例如磷脂酰胆碱、磷脂酰乙醇胺、磷脂酰丝氨酸、磷脂酰肌醇、磷脂酰甘油、磷脂酸、溶血磷脂、卵磷脂或大豆磷脂或其组合。可以对磷脂进行盐化或脱盐、氢化或部分氢化或者所述磷脂可以为天然的、半合成的或合成的磷脂。这些表面活性剂的详细描述可以参见雷明顿药物科学(第 18 版，1990，Mack Publishing Co. PA)和药品工业的理论和实践(Lachman 等，1986).

以下实施例进一步解释并说明本发明：

实施例 1

压缩液化气中的水不溶性化合物的相行为

为了评估在液化气中是否可以将一种特定的水不溶性化合物从其溶液配制成含水的亚微颗粒悬浮体，需要对测试药物的溶解性进行测量。

为制备具有恒定摩尔组成的溶液，将定量的药物(非诺贝特)加入恒定体积的观察池。温度保持恒定在 60℃。通过泵入压缩液化气进入观察池使压力在 1300-4000 psi 范围内变化。通过目测观察固体药物开始溶解时的压力来确定相行为。非诺贝特在液化的二氧化碳、丙烷和乙烷中的溶解性列于表 I。当在溶剂中的溶解性大于 1%(w/w)时，可以由这些溶剂来制备微细颗粒。

表 I 非诺贝特在 60℃的液化二氧化碳、丙烷和乙烷中的溶解性

液化气体	压力(psi)	溶解性(%, w/w)
二氧化碳	1800	0.01
	2000	0.08
	2800	1.4
丙烷	1500	2.5
	2000	2.3
乙烷	1300	0.016
	2000	0.79
	3000	1.80
	4000	1.90

实施例 2

通过 RELGS 法形成非诺贝特微粒

将加压至 3000psi 的、含有非诺贝特(2g)、类脂 E-80(0.2g)、吐温-80(0.2g)的液体二氧化碳溶液膨胀通过 50mm 的孔板进入大气压和室温(22℃)下的水中。得到平均粒度约 200nm 的非诺贝特的微细悬浮体。使用亚微粒度仪-Autodilute Model 370(NICOMP Particle Sizing Systems, Santa Barbara, CA)，通过光子相关的光谱(photon correlation spectroscopy)进行粒度测试。该仪器提供数量重量、强度重量和容积

重量的粒度分布以及多形态的粒度分布(如果存在的话).

实施例 3

用具有 63.5mm 内径的 PEEK 毛细管制成微细喷嘴. 该 PEEK 喷嘴用 M-100 Minitight 阳螺母进行固定并且连到 Upchurch SS20V 联合体上, 该联合体通过适当大小的 Swagelok™ 牌连接件进一步连接至 1/4 英寸的高压歧管上. 除了 PEEK 管外, 所有其它元件均由 316 不锈钢制成. 通过 1/4 英寸的高压歧管, 将水不溶性物质的高压液化气溶液(>1000 psig)导入 63.5mm 的 PEEK 喷嘴, 从而膨胀进入水介质中. 向液化气溶液容器中注入 1g 环孢菌素和 0.2g 吐温-80. 用 5000psig 的二氧化碳填充所述容器并加热至约 24℃. 静置所述容器约 20 分钟以完成溶解并达到平衡. 在 6000psi 下, 使用 Avestin Emulsiflex C50 均化器(Avestin Inc, Ottawa, Canada), 单独对在 5.5%甘露醇溶液中的 2%(w/w)卵磷脂(来自 Lipoid GmbH 的 Lipoid E80)悬浮体均化 15 分钟, 生成清澈分散体. 在均化前, 用氢氧化钠水溶液调节磷脂悬浮体的 pH 至 8.0. 使 24℃、5000psig 下放置的环孢菌素和吐温-80 的二氧化碳溶液膨胀进入卵磷脂的水分散体中. 以极快的速度(约 3 分钟)获得约 23 纳米粒度的透明水悬浮体(参见图 1). 这个实施例通过将几个这样的 PEEK 喷嘴置于歧管内, 同时膨胀进入含有适量水性介质的储罐中, 从而提供一个简单的可放大的方法. PEEK 喷嘴被认为是惰性的并且很便宜. 制造这种喷嘴非常简单, 不到 10 分钟即可制成.

实施例 4

由 RELGS-H 法形成环孢菌素微粒

制备含有甘露醇(5.5%)、Lipoid E-80(2%)和吐温 80(2%)的水悬浮体. 还制备环孢菌素的液化气溶液并保持在 2000psig 和 60℃下. 通过 63.5mm PEEK 喷嘴, 使该溶液膨胀进入水悬浮体中. 以这种方法制备约 3g 环孢菌素的悬浮体. 在 6000psig 下, 均化所得悬浮体 8 次. 均化后, 用亚微粒度仪-Autodilute Model 370(NICOMP Particle

Sizing Systems, Santa Barbara, CA)进行测量，最终的平均粒度为 86 纳米，99%为 150 纳米(参见图 2).

虽然本发明是参照目前认为最实用并且优选的实施方案进行描述，但应该理解的是本发明并不受限于所公开的实施方案，但另一方面，本发明覆盖包括在所附权利要求的精神和范围内的各种变化和相当的配置.

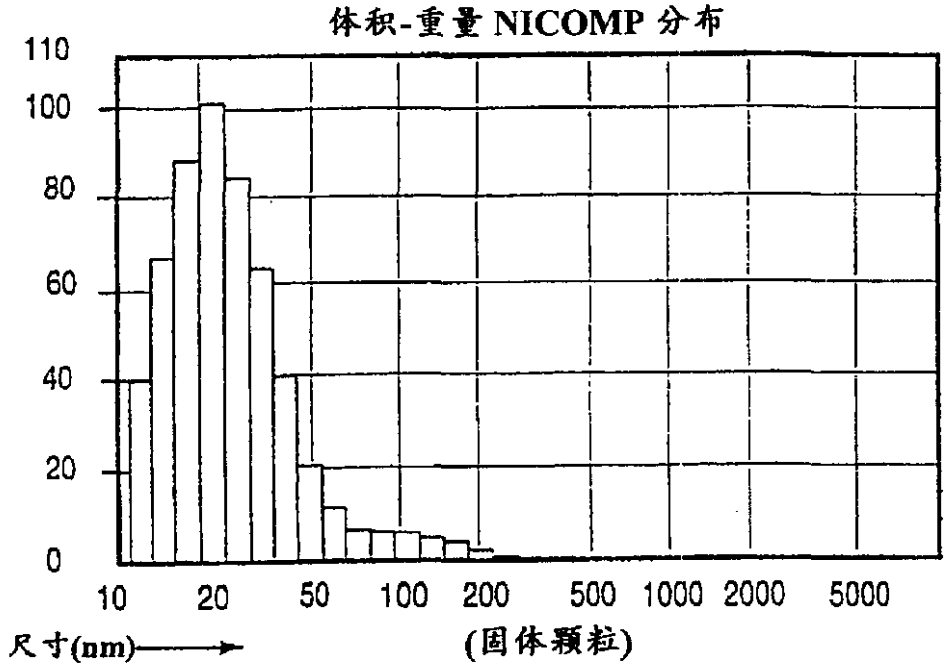
01.02.14

说 明 书 附 图

体积-重量 NICOMP 分布分析
(固体颗粒)

NICOMP 综述：
峰值 1 ： 平均直径=22.7 nm　　体积： 100.00%

相对体积



平均直径=30.1 nm　　Pit 误差=3.897　　残余=2.015

NICOMP 比例参数：
最小直径=10.0 nm　　图大小(Plot Size)=36
平滑度=4　　图范围(Plot Range)=1000

运行时间	=0 小时 7 分钟 26 秒	波长	=632.8 nm
计数频率	=301 · Khz	温度	21 ℃
通道#1	=267.8 K	粘度	0.933 cp
通道宽度	=11.0 uSec	引用索引(Index of Ref.)	1.333

高斯综述：

平均直径	=37.7 nm	卡方值	=36.603
标准偏差	=22.1 nm (58.6%)	基线调整	=0.016%
Var'n 系数	=0.586	平均微分系数	=1.24E-07 cm^2/s

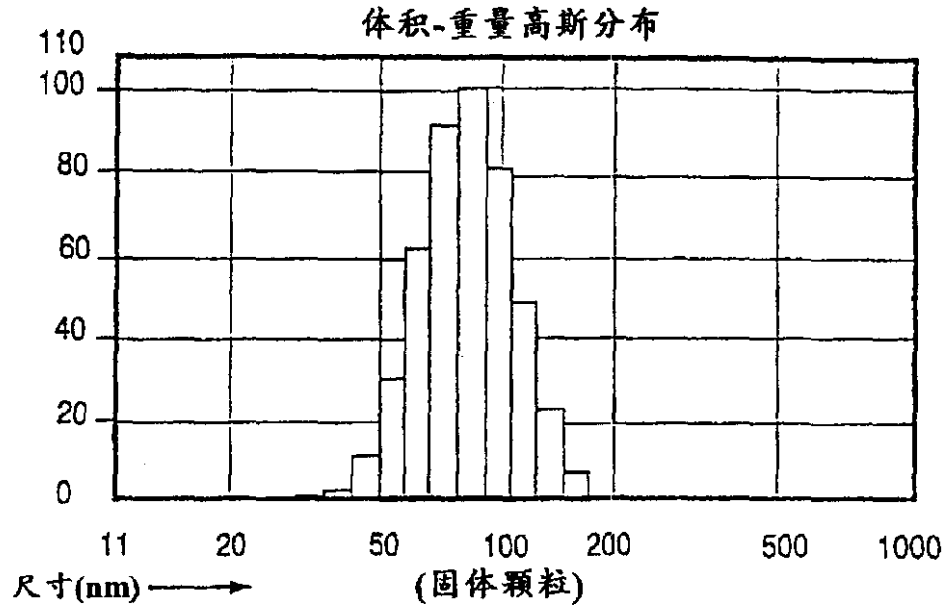
图 1

体积-重量高斯分析
(固体颗粒)

高斯综述：

平均直径	=86.0 nm	卡方值	=0.218
标准偏差	=24.9 nm (29.0%)	基线调整	=0.012%
Var'n 系数	=0.290	平均微分系数	=5.37E-08 cm^2/s

相对体积



累积结果：
25%分布<62.68 nm
50%分布<76.22 nm
75%分布<93.05 nm
99%分布<151.39 nm

运行时间	=0 小时 7 分钟 12 秒	波长	=632.8 nm
计数频率	=324 Khz	温度	21 ℃
通道#1	=310.1 K	粘度	0.933 cp
通道宽度	=12.0 uSec	引用索引	1.333

图 2