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Menetelmä ja laite nanopartikkeleiden valmistamiseksi

Förfarande och anordning för framställning av nanopartiklar

A method and a device for producing nanoparticles

(57) Tiivistelmä - Sammandrag - Abstract

Keksintö koskee menetelmää ja laitetta orgaanisten yhdisteiden nanopartikkeleiden valmistamiseksi, erityisesti käyttäen hyväksi kontrolloitua kaksivaiheista paineistettujen
liuosten laajenemista.

The invention relates to a method and a device for producing nanoparticles of organic substances, in particular by controlled two step expansion of pressurized solutions.

A METHOD AND A DEVICE FOR PRODUCING NANOPARTICLES

FIELD

The invention relates to a method and a device for producing nanoparticles of organic substances, in particular by controlled two-step expansion of pressurized solutions.

BACKGROUND

Nanoparticles exhibit size-dependent physical and chemical properties, such as reduced melting point and increased reactivity and solubility. These special properties of nanoparticles are often due to their large surface area. The increase in solubility of nanosized material is a thermodynamic effect that results from the increased chemical potential at a curved surface.

In a typical RESS (rapid expansion of supercritical solutions) process, supercritical fluid is used to dissolve solid material under high pressure and temperature, thus forming a homogeneous supercritical phase. Thereafter, the solution is expanded through a nozzle, and small particles are formed. At the rapid expansion point right at the opening of the nozzle, there is a sudden pressure drop that forces the dissolved material to precipitate out of the solution. The crystals that are instantly formed enclose a small amount of the solvent that, due to the expansion, changes from supercritical fluid to its normal state, thus breaking the crystal from inside-out. The particles that are formed this way may have a diameter of a few hundreds of nanometers.

Supercritical fluid processing techniques have shown promise in production of small particles of water-insoluble materials. For example WO 97/14407 and WO 99/65469 describe processes that generate submicron-size particles of biologically useful materials through the use of supercritical or compressed fluid processing techniques. However, these processes produce particle suspensions containing a substantial fraction of drug particles larger than 100 nm. Substantially smaller particles would be advantageous for medical applications. The process was further developed in WO 2006015358 that discloses a method to prepare homogenous aqueous suspensions of nanoscale drug particles with the aid of stabilizing agents. According to the process disclosed in WO 2006015358, all the formed particles are smaller than 100 nm, and standard deviation of particle size was less than 15 nm.

WO 97/31691 discloses a method and apparatus for particle precipitation and coating, wherein the precipitable substance is in contact with a supercritical antisolvent together with an energizing gas stream to generate focused high frequency sonic waves in the antisolvent to break the particles into smaller ones. The size or the particles obtained using the technology was 0.1 - 10 μm.

US 7,815,426 discloses an apparatus and method for preparing nanoparticles wherein a suspension of an organic substance is passed through a micro flow channel, and the organic substance is irradiated with a laser beam.

Since nanoparticles find many potential applications and, since there is a limited number of processes to produce them, there is a need to develop new methods to prepare such particles.

SUMMARY

The present invention is based on the observation that less than 20 nm nanoparticles can be obtained, in contrast to the RESS process of prior art, by using a two-step gradient pressure reduction process that creates conditions for controlled expansion of supercritical solutions.

According to one aspect the present invention concerns a new method for producing nanoparticles of an organic substance, the method including:

- admixing the organic substance and a supercritical fluid to form a mixture at a first pressure,
- decreasing the first pressure gradually to a second pressure in such a manner that a flow of the mixture is formed and nucleation of the organic substance in the mixture is initiated, and
- decreasing the second pressure to a third pressure, in such a manner that adiabatic solidification of the fluid of the mixture, comprising the nucleated organic substance, is initiated.

According to another aspect, the present invention concerns a new device for producing nanoparticles of an organic substance, the apparatus including:

- a pressure chamber for a mixture of the organic substance and a supercritical fluid,
- an outlet tube connecting the pressure chamber to a collection chamber, the outlet tube being provided with

- a pressure controlling means configured to control pressure of the mixture within the outlet tube, and

- a first nozzle configured to allow expansion of the mixture to the collection chamber,

wherein the device further includes one or more second nozzles, for one or more second fluids, the one or more second nozzles being configured to allow adiabatic solidification of the one or more second fluids, and to allow subjecting the mixture expanding from the first nozzle to the solidifying one or more second fluids.

According to another aspect the present invention concerns use of the device of the present invention for producing nanoparticles of medicaments.

According to another aspect the present invention concerns particles of piroxicam obtainable by a method according to any of claims 1 to 9.

Further aspects of the present technology are described in the accompanying dependent claims.

Exemplifying and non-limiting embodiments of the invention, both as to constructions and to methods of operation, together with additional objects and advantages thereof, are best understood from the following description of specific exemplifying embodiments when read in connection with the accompanying drawings.

The verbs “to comprise” and “to include” are used in this document as open limitations that neither exclude nor require the existence of un-recited features. The features recited in the accompanied depending claims are mutually freely combinable unless otherwise explicitly stated. Furthermore, it is to be understood that the use of "a" or "an", i.e. a singular form, throughout this document does not exclude a plurality.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows a schematic illustration of a device for the preparation of nanoparticles according to an exemplary, non-limiting embodiment of the invention,

figure 2 shows piroxicam particles prepared according to a method of prior art (left: particle size 5 µm; right: particle size 12 µm),

figure 3 shows exemplary piroxicam particles prepared according to a method of the present invention (top left: particle size 50 nm; top right: particle size 200 nm; bottom: particle size 16 nm),

figure 4 shows exemplary piroxicam particles and their particle size distribution prepared according to a method of the present invention,

figure 5 shows exemplary piroxicam nanoparticles prepared according to an apparatus of the present invention, and

figure 6 shows exemplary dissolution profiles piroxicam particles prepared according to a method of the present invention and according to a method or prior art.

DESCRIPTION

The present invention for producing nanoparticles of organic substances, preferably with narrow size distribution, is based on a two-step gradient pressure reduction process that creates conditions for controlled expansion of supercritical mixtures. The process combines controlled flow, controlled pressure reduction, and preferably also particle collection. The pressure gradient process can also be generated by using a tapered tube with increasing cross section towards the orifice. An exemplary device suitable for the preparation of nanoparticles according to the present invention is shown in Figure 1.

According to one embodiment of the present invention, a mixture of the organic substance in a supercritical fluid is allowed to expand from a pressure chamber (1) to an outlet tube (2) equipped with pressure controlling means (3). The first pressure reduction step takes place in the outlet tube (2) connecting the pressure chamber (1) to the collection chamber (5). A pressure controlling means for instance a needle valve of the outlet tube, releases the substance solution through a first nozzle (4) to the collection chamber. The flow rate inside the outlet tube is kept low to ensure a controlled, preferably laminar or substantially laminar flow of the mixture. The pressure is allowed to decrease gradually inside the outlet tube causing supersaturation of the substance in the fluid, which initiates the nucleation process. Having the pressure reduction controlled and gradual, keeps the nucleate formation process slow which is important in order to prevent concentration of nucleates and blocking of the outlet tube. This slow nucleate formation together with the controlled laminar flow or at least substantially laminar flow in the outlet tube inhibits unwanted growth of the formed nucleates.

According to an embodiment liquid CO_2 is transferred from a container (8) to a pressure chamber (1) using a high pressure pump (9). According to an exemplary embodiment CO_2 is pumped to the pressure required to form supercritical fluid (>74 bar) and temperature (> 300 K). The substance, such as a drug molecule is introduced to the pressure chamber

followed by admixing with the supercritical CO_2 ($scCO_2$) to form a supercritical fluid. Proper mixing and thus formation of a homogenous mixture can be ensured by using e.g. a magnetic mixer (10). The system pressure can be monitored with an internal pressure gauge of the pressure pump whereas the temperature can be monitored with a thermocouple and/or a thermometer. The pressure chamber is preferably equipped with temperature controlling means and pressure controlling means and is coated with an insulating material. The device is equipped with a collection chamber (5) which preferably is insulated. The pressure in the collection chamber is below the pressure in the pressure chamber when the device is operated.

An exemplary device used for preparation of nanoparticles of piroxicam included an outlet tube (2) of length and internal diameter of the outlet tube 60 cm, and 2 mm, respectively. The first pressure reduction takes place in the needle valve (3). An exemplary flow rate was 24 mL/min. It is obvious for a skilled person that the flow rate and the decrease of the pressure required for nucleation initiation within the outlet tube depends on the nature of the organic substance and supercritical fluid used, temperature, as well as the construction of the device.

The second pressure reduction step takes place at the first nozzle (4). As the volume of the supercritical fluid such as $scCO_2$ increases, the pressure decreases, and a gaseous phase is formed. This step is controlled both by the nozzle in the device and by adiabatic dry ice formation. Dry ice formation around nucleates of the substance controls particle growth and prevents aggregation of the nucleates. According to a preferable embodiment ultrasonic agitation of the first nozzle is also performed. This further prevents aggregation of the nucleates and controls the particle growth.

The particle size of organic substances obtained by the method of the present invention is 200 nm or less, preferably less than 100 nm, and more preferably less than 50 nm, and most preferably less than 20 nm.

As defined herein a “nanoparticle” is a particle whose average diameter is 200 nm or less.

As defined herein an “organic substance” is a molecule containing carbon, excluding carbon containing alloys, and relatively small number of carbon-containing compounds such as metal carbonates and carbonyls, simple oxides of carbon and cyanides, as well as allotropes of carbon and simple carbon halides and sulfides which are considered inorganic. Exemplary organic substrates used in the present technology are biologically

active materials including medicaments and their pharmaceutically acceptable organic and inorganic salts.

A non-limiting list of exemplary classes of biologically active materials that may be of interest to the technology include analgesics, antagonists, anti-inflammatory agents, anthelmintics, antianginal agents, antiarrhythmic agents, antibiotics (including penicillins), anticholesterols, anticoagulants, anticonvulsants, antidepressants, antidiabetic agents, antiepileptics, antigonadotropins, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, antipsychotic agents, immunosuppressants, antithyroid agents, antiviral agents, antifungal agents, anxiolytic sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, anti-cancer agents, cardiacinotropic agents, contrast media, corticosterioids, cough suppressants (expectorants and mucolytics), diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immunosuppressive and immunoactive agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radiopharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anorexics, sympathomimetics, thyroid agents, vasidilators, neuron blocking agents, anticholinergic and cholinomimetic agents, antimuscarinic and muscarinic agents, vitamins, and xanthines.

Exemplary medicaments suitable for the method of the present technology are entacapone, esomeprazole, atorvastatin, rabeprazole, piroxicam and olanzapine. An exemplary medicament is piroxicam (4-hydroxy-2-methyl-N-(2-pyridinyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide).

The supercritical fluid is preferably CO_2, but also other supercritical fluids or their mixtures can be used. The organic substances to be nanosized are dispersed or dissolved in a proper medium, preferably into a supercritical fluid or a near critical fluid. The medium employed in the disclosed process can generally be any of a number of liquefied compressed gases and their mixtures known to the art. These include but are not limited to gaseous oxides such as nitrous oxide; water; 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, dichlorofluoromethane,

difluorochloromethane, and fluorocarbons such as trifluoromethane; and elemental liquefied gases such as xenon. Optionally, the medium can include mixtures of one or more suitable materials. In general, the biocompatibility of the medium is not an issue in the disclosed process, as the supercritical medium will generally be separated completely after expansion, with the gas leaving the system or being collected for recycling.

It is to be understood that also near-supercritical form media can be used. The medium, advantageously a supercritical fluid, can act as a solvent or as an antisolvent.

Figure 2 shows particles of a medicament (piroxicam) prepared according to a prior art method, and Figures 3-5 show exemplary particles of the same drug prepared according to the present invention. As seen in the figures, a significant reduction in particle size can be achieved by the present method.

In an exemplary process using the device in Figure 1 for producing nanoparticles of piroxicam, formation of dry ice, including nucleates of piroxicam, started ca 2-3 cm from the first nozzle. The average particle size in this case was 200 nm. According to a preferable embodiment, solidifying CO_2 containing particles of the sample substance is subjected to another flow of CO_2. The additional flow stops or at least reduces the growth of the particles. Also the collection of the sample substance is simplified.

Accordingly, it is preferable to further enhance the solidification of the fluid, such as dry ice formation, by an additional flow of one or more second fluids in the proximity of the first nozzle (4). This can be achieved by one or more additional nozzles, *i.e.* second nozzles (6) equipped with a fluid inlet. The distance and the angle of the second nozzles are preferably chosen such that the formation of the solidifying fluid such as dry ice from these nozzles takes place earlier than the formation of the solidifying fluid, such as dry ice, expanding through the first nozzle. The second nozzle can be concentric with the first one. The additional solidifying fluid such as dry ice prevents the increase in particle size of the sample substance. Furthermore, since the formed solid dispersion includes a significant amount of solid fluid, such as dry ice, aggregation of the particles of the sample substance is less prominent. Although CO_2 is a preferable second fluid, also other fluids and their mixtures can be used.

According to one embodiment the method includes collection of the nanoparticles, e.g. on a filter (11) located in the collection chamber.

According to another embodiment the method further includes flushing the collection chamber (5), preferably the filter (11) including nanoparticles of the sample substance with dry nitrogen from a second container (12) via a third nozzle (7). Inert nitrogen prevents particle aggregation as the solidified fluid such as dry ice sublimates. It also prevents moisturizing of the particles of the sample substance. Also other inert gases, such as argon can be used for this purpose. The particles remain separate and can be used in drug formulations or stored as a solid dispersion e.g. in dry ice or in liquid nitrogen.

According to another embodiment, the method is used to optimize the solid state form of the substance and to produce advantageous polymorphic forms or mixed crystals of the substance. According to one embodiment the method is intended for manufacture of pharmaceutical excipients, active drug substances and drug/drug, drug/excipient and excipient/excipient mixtures. According to one embodiment the expients are selected from antiadherents, binders, coatings, disintegrants, fillers, flavors, colors, lubricants, glidants, sorbents, preservatives, sweeteners, tracers and ultrasonic or photo acoustic enhancers.

According to one embodiment the present technology is used to produce multi-functional nano-sized colloidal particles (MF colloidal particles), where different components are included in each particle and/or where a significant fraction of particles contains various components in equal ratios. The MF colloidal particles may be partly or totally crystalline and/or amorphous. According to an embodiment the MF particles contain one or more active components, and one or more supportive components that serve to improve machineability, solubility, wetting, dissolution rate, uptake, chemical and/or physical stability, as well as various powder properties, e.g. flowability and biological activity.

According to another embodiment the method is used to produce multifunctional particles including the active substance and various expients.

According to another embodiment the present invention concerns a device for producing nanoparticles of a substance, the device including:

- a pressure chamber (1) for a mixture of the organic substance and a supercritical fluid,
- an outlet tube (2) connecting the pressure chamber to a collection chamber (5), the outlet tube being provided with
- a pressure controlling means (3) configured to control pressure of the mixture within the outlet tube, and

- a first nozzle (4) configured to allow expansion of the mixture to the collection chamber, and
- one or more second nozzles (6), for one or more second fluids, the one or more second nozzles being configured to allow adiabatic solidification of the one or more second fluids, and to allow subjecting the mixture expanding from the first nozzle to the solidifying one or more second fluid.

The first nozzle of the device according to the present invention are constructed from a material generally used as nozzle materials. Exemplary common materials are various grades of stainless steel. Other exemplary materials are titanium, sapphire, fused quartz, graphene, carbon nanotubes, silicone single crystals, diamonds and their assemblies. The diameter, shape and aspect ratio of the nozzle can be chosen according to the desired flow. According to one embodiment the nozzle includes adjusting means to alter the aspect ratio and/or to modify the geometry of the one or more nozzle channels.

According to one embodiment device includes a nozzle actuation means configured to actuate the first nozzle by focused or unfocussed laser light or high frequency ultrasound. The actuation avoids clogging of the nozzle by the substance. According to a preferable embodiment, the first nozzle is connected to a piezo actuator configured to actuate the exit surface or the external proximity of the first nozzle at a frequency of 1 MHz or higher. The nozzle actuation means is not shown in Figure 1.

The first nozzle (5) can be any expansion nozzle as is generally known in the art. For example, the nozzle can be a specifically designed and constructed orifice. In one embodiment, the first nozzle is a fused-silica capillary held within stainless steel tubing. According to a preferable embodiment, the first nozzle has an internal diameter between 1 and 100 µm and an aspect ratio (L/D) of at least 5.

The device according to the present invention includes one or more second nozzles for one or more second fluids. The one or more second nozzles (7) are constructed from a material generally used as nozzle materials. An exemplary second nozzle is a ruby nozzle including a 300 µm orifice. According to a preferable embodiment the one or more second nozzles are produced by 3D printing. The advantage of the 3D printing is that the structure of the nozzle can be designed according to the construction of the device and the demands of the organic substance.

The disclosed process can generally utilize any liquefied compressed gases known to the art. These include but are not limited to gaseous oxides such as 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, and fluorocarbons such as trifluoromethane and elemental liquefied gases such as xenon. Optionally, the process can include mixtures of one or more materials. In general, biocompatibility is not an issue in the disclosed process, as the supercritical fluid will generally completely evaporate and leave the system or be collected for recycling.

According to a preferable embodiment the device according to the present invention further includes a third nozzle (8) configured to fluid the collection chamber with an inert gas. The technical effect of the third nozzle is disclosed above.

EXPERIMENTAL

Piroxicam (Hawkings Inc., U.S.) and CO_2 (purity ≥ 99.8% AGA, Finland) were used for particle production. Phosphate buffer (100 mM; pH 7.2) used in the dissolution tests was prepared according to the European Pharmacopoeia (Ph. Eur. 7th ed.). All the reagents were used as received and were of analytical grade.

Comparative example

Traditional RESS devices were tested with piroxicam for reference. Both a laboratory scale device and a pilot scale RESS device was employed. In the laboratory scale device, a 200 bar pressure and 60°C temperature in the pressure chamber as well as a RESS orifice with diameter 100 µm were used. No collection chamber was used. In the pilot scale device particles were produced at 200-230 bar and 60°C with a collection chamber at 55 bar and 31°C.

The average particle size of particles prepared with the laboratory scale device and with the pilot scale device were 5 µm and 12 µm, respectively. Examples of the particles are shown in Figure 2.

Example 1

The system's main components were a high pressure pump (SFT-10, Supercritical Fluid Technologies, Inc., USA), a custom high pressure chamber, a heater/mixer (MR 2002,

Heidolph, Germany), a ruby nozzle (150 µm orifice) and a collection chamber. The pressure chamber was loaded with a sample substance (piroxicam; 300 mg, saturated) followed by liquid CO_2. Pressure and temperature was increased to 200-310 bar and 60 °C, respectively. A magnetic mixer (1500 rpm) ensured proper dissolution of the sample substance and formation of a homogenous mixture. Supersaturation state was obtained within 30 min.

The first pressure reduction step was allowed to take place in the needle valve of the outlet tube connecting the pressure chamber to the collection chamber. The sample substance was allowed to release into the collection chamber through a nozzle. The flow rate inside the outlet tube (length 60 cm, diameter 2 mm) was kept at 24 mL/min with the aid of a needle valve (SS-3HNTF2, Swagelok) to ensure laminar flow. Accordingly, the pressure was allowed to decrease gradually to a non-supercritical state to initiate the nucleation of the sample substance within the outlet tube.

The second pressure reduction step was allowed to take place at the exit nozzle (i.e. the first nozzle), As the CO_2 volume was increased, a gaseous CO_2 phase was formed. This step was controlled by adiabatic dry ice formation. Dry ice formation around the nucleates controlled particle growth and prevented aggregation of the nucleates.

Dry nitrogen was used to flush the collection chamber. Inert N_2 prevented particle aggregation as dry ice sublimates. The particles remained separate. The particles were stored as a solid dispersion and dry ice. Finally, they were collected as dry powder of pure nanoparticles after sublimation of CO_2. The chemical integrity and polymorphism of the nanoparticles were evaluated with Fourier transformed infrared spectroscopy (FTIR). FTIR spectra were recorded at room temperature using a Vertex 70 (Bruker, USA) with a horizontal attenuated total reflectance (ATR) accessory (MIRacle, PIKE Technologies, USA) between 4000-650 cm^{-1}. This provided a 4 cm^{-1} resolution when using the OPUS 5.5 software.

50 nm particles were collected with a preparation process of 150-250 bar and 70°C in the pressure chamber using a flattened tube nozzle. Examples of particles are shown in Figure 3 (top left)

200 nm particles were prepared reproducibly with a steady process with 150-330 bar and 60-90 °C in the pressure chamber featuring a Ø=150 µm ruby nozzle. With both processes the flow in the outlet tube from the pressure chamber to the nozzle was controlled and kept

near laminar. A needle valve was used to control the flow and after the adiabatic dry ice formation at the nozzle was established. Examples of particles are shown in Figure 3 (top right)

16 nm particles were formed in conditions where the CO_2 flow from the outlet tube was kept very slow and the pressure chamber was used at 200 bar and 60°C. A needle valve was used to control the flow and the particles were collected on glass slides. Examples of particles are shown in Figure 3 (bottom).

Example 2. The effect of second fluid

a) Piroxicam in $scCO_2$ was allowed to expand through the first nozzle to the collection chamber at 330 bar and 72 °C. The particle size was 500 nm.

b) Piroxicam in $scCO_2$ was allowed to expand through the first nozzle to the collection chamber at 330 bar and 72 °C, and additional CO_2 through the second nozzle was subjected to the forming particles of piroxicam. Particle size obtained was 200 nm. Examples of particles are shown in Figure 4.

Determination of particle size

Particle size and morphology of the particles were examined by scanning electron microscopy (SEM). Piroxicam nanoparticles and bulk (reference) piroxicam were imaged with a Quanta™ 250 FEG (FEI Inc., U.S.). Samples were collected on a metal net residing on a carbon-coated double-sided tape. Samples were sputter-coated with a 5 nm thin layer of platinum (Q150T Quomm, Turbo-Pumped Sputter Coater, China). The coated samples were imaged in 9.85 x 10^{-4} Pa pressure, with 30 µm aperture, 10kV, ≤ 200 nA, and a 2.5 nm spot size. Each image was obtained in ca 5 minutes. The particle size was determined by diameter measurements and analysis with the ImageJ freeware (National Institutes of Health, USA).

The average diameter of the nanoparticles prepared as disclosed in Example 1 was 210 nm ± 59 nm (n=300). The size distribution of the nanoparticles was narrow (Fig. 5) and the formed particles were round with a slightly elongated shape and with no visible fracture planes or aggregates. The smallest particles obtained according to the method were 16 nm.

Drug release tests

Drug release tests for piroxicam nanoparticles and bulk (reference) piroxicam were done to investigate the effect of particle size on the dissolution rate. The tests were conducted in glass vials under heating (37.0 ± 0.5 °C) and stirring (400 rpm) (H+P Labortechnik AG, Multitherm, Germany). Samples were placed in a gelatin capsule and anchored with an iron wire to prevent the capsule from surfacing.

The capsule was then placed in glass vials containing phosphate buffer (50 mL; pH 7.2). Aliquots (1 mL) were taken at the time points ranging from 1 min to 48 hours. Drug release tests were conducted in triplicate. Samples were analyzed with high performance liquid chromatography (HPLC Thermo System Products, Agilent 1200 Infinity Series, Agilent Technologies, Germany) using a Discovery® C18 (Supelco Analytical, U.S.) column with guard column and a flow rate of 1 mL/min. The mobile phase was 60:40 (v/v) acetonitrile and 0.05 % trifluoroacetic acid. The UV detection of piroxicam was set to 333 nm with a retention time of 2.9 min and total run time of 4 min at 30 °C. A standard curve for BSA quantification was made from piroxicam concentrations of 0.1 - 25 µg/mL (R^2 = 0.999).

Figure 5 illustrates the dissolution profiles of the nanoparticles and bulk piroxicam. The dissolution rate of the bulk piroxicam agreed and exceeded that reported in the literature [Lai et al. 2011 doi: 10.1016/J.EJPB.2011.07.005]. The dissolution rate of the nanoparticles was twice that of the dissolution rate of bulk piroxicam. The gelatin capsules caused a lag time of 1-2 min in the dissolution rate profiles. All samples completely dissolved within 24 hours. Nanoparticles were completely dissolved from the gelatin capsules within one hour from the beginning of the test.

The non-limiting, specific examples provided in the description given above should not be construed as limiting the scope and/or the applicability of the appended claims.

CLAIMS

1. A method for producing nanoparticles of an organic substance, the method comprising

- admixing the organic substance and a supercritical fluid to form a mixture at a first pressure,
- decreasing the first pressure gradually to a second pressure so as a flow of the mixture is formed and nucleation of the organic substance in the mixture is initiated, and
- decreasing the second pressure to a third pressure, so as adiabatic solidification of the fluid of the mixture, comprising the nucleated organic substance, is initiated.

2. The method according to claim 1, wherein the flow is substantially laminar.

3. The method according to claim 1 or 2, wherein decreasing the second pressure to the third pressure is carried out by expanding the mixture from the second pressure to the third pressure through a first nozzle.

4. The method according to claim 3 further comprising actuating the first nozzle with laser light or ultrasound of frequency of at least 1 MHz.

5. The method according to any of claims 1 to 4 further comprising

- obtaining one or more second fluids,
- allowing the one or more second fluids to expand so as adiabatic solidification of the one or more second fluids is initiated, and
- subjecting the mixture comprising the nucleated organic substance to the solidifying one or more second fluid.

6. The method according to any of claims 1-5, wherein the supercritical fluid comprises carbon dioxide.

7. The method according to claim 5 or 6, wherein the one or more second fluids comprises carbon dioxide.

8. The method according to any of claims 1 to 7, further comprising collection of the nanoparticles.

9. The method according to any of claims 1 to 8 further comprising flushing the nanoparticles with an inert gas.

10. A device for producing nanoparticles of an organic substance, the device comprising:

- a pressure chamber (1) for a mixture of the organic substance and a supercritical fluid,

- an outlet tube (2) connecting the pressure chamber to a collection chamber (5), the outlet tube being provided with

  - a pressure controlling means (3) configured to control pressure of the mixture within the outlet tube, and

  - a first nozzle (4) configured to allow expansion of the mixture to the collection chamber,

characterized by that the device further comprising one or more second nozzles (6), for one or more second fluids, the one or more second nozzles being configured to allow adiabatic solidification of the one or more second fluids, and to allow subjecting the mixture expanding from the first nozzle to the solidifying one or more second fluid.

11. The device according to claim 10, further comprising actuation means configured to actuate the first nozzle and/or an external volume close to the exit surface the first nozzle with laser light or ultrasound of at least 1 MHz.

12. The apparatus according to claim 10 or 11, further comprising a third nozzle (7) within the collection chamber, for a third fluid, the third nozzle being configured to subjecting the third fluid on the nanoparticles in the collection chamber, and wherein the third fluid is an inert gas.

13. Use of the device according to any of claims claim 10 to 12 for producing nanoparticles of medicaments.

14. Nanoparticles of piroxicam obtainable by a method according to any of claims 1 to 9.

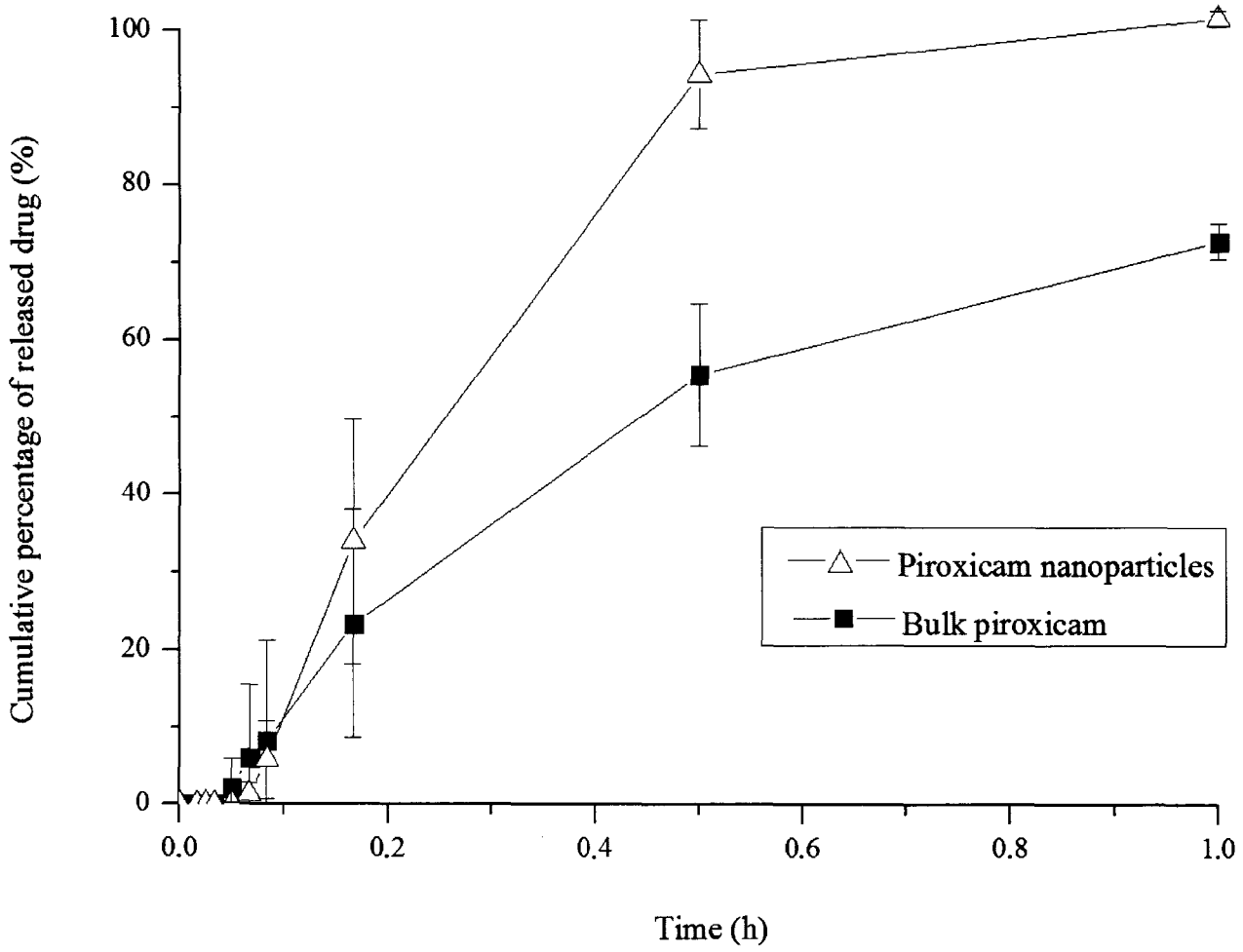
Cumulative percentage of released drug (%)
100
80
60
40
20
0
0.0
0.2
0.4
0.6
0.8
1.0
Time (h)
Piroxicam nanoparticles
Bulk piroxicam

Figure 6

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PATENT APPLICATION No.	**CLASSIFICATION**	
20140266	IPC ***A61K 9/14*** (2006.01) ***A61K 9/51*** (2006.01) ***B01J 2/04*** (2006.01) ***B01D 9/02*** (2006.01) ***A61K 9/16*** (2006.01) *B01J 3/00* (2006.01)	CPC **A61K 9/5192** **B01J 2/04** **B01D 9/02** **A61K 9/1682** B01J 3/008

PATENT CLASSES SEARCHED (classification systems and classes)
IPC: A61K, B01D, B01J

DATABASES CONSULTED DURING THE SEARCH
EPO-Internal, WPI, XP3GPP, XPAIP, XPESP, XPI3E, XPIEE, XPIETF, XPIOP, XPIPCOM, XPMISC, XPOAC, XPRD, XPTK, BIOSIS, COMPDX, EMBASE, INSPEC, MEDLINE, PUBCOMP, PUBSUBS, TDB, NPL

DOCUMENTS CONSIDERED TO BE RELEVANT

Category*)	**Bibliographic data on the document and relevant passages**	**Relevant to claims**
X	US 2006153921 A1 (CHATTOPADHYAY PRATIBHASH [US] et al.) 13 July 2006 (13.07.2006) figure 1; paragraphs [0018], [0027], [0029], [0033], [0038]–[0040], and [0044]–[0046]	1-4, 6, 8-9
X	Wen et al. 'Application of an Improved RESS Process for Atractylodes Macrocephala Koidz Volatile Oil Liposomes Production' in the Proceedings of 4th International Conference on Bioinformatics and Biomedical Engineering (iCBBE), pages 1–4, June 2010, doi: 10.1109/ICBBE.2010.5517532 abstract; section II.C; figure 1	10-13

Continued on the next sheet ☒

*) X Document indicating that the invention is not novel or does not involve an inventive step with respect to the state of the art.
Y Document indicating that the invention does not involve an inventive step with respect to the state of the art if combined with one or more other documents in the same category.
A Document representing the general state of the art.

O Document referring to disclosure through lecture, use or other non-written means.
P Document published prior to the filing date but not prior to the earliest priority date.
T Document published after the filing date or priority date and illustrating the principle or theory underlying the invention.
E Earlier patent or utility model application that either is Finnish or designates Finland published on or after the filing date (priority date).
D Document that is mentioned in the application.
L Document which may throw doubts on priority claim(s), is cited to establish the publication date of another citation or is referred to for some other reason.

& Document member of the same patent family.

This document has been electronically signed. **Further information given in the annex** ☐

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DOCUMENTS CONSIDERED TO BE RELEVANT, CONTINUED		
Category*)	**Bibliographic data on the document and relevant passages**	**Relevant to claims**
X	US 2004071783 A1 (HANNA MAZEN HERMIZ [GB] et al.) 15 April 2004 (15.04.2004) figures 1 and 3–4; paragraphs [0076]–[0081] and [0095]	10-13
X	US 2006210622 A1 (PACE GARY W [US] et al.) 21 September 2006 (21.09.2006) claims 1 and 10	14
X	Ikeda et al. 'Enhanced skin permeation of piroxicam and pranoprofen induced from nanoparticles dispersed in propylene glycol aqueous solution', EMBASE/Elsevier abstract, 2012. The full article published in Journal of Drug Delivery Science and Technology Volume 22, Issue 2, pages 131–137, 2012. abstract	14
A	Hezave et al. 'The effects of RESS parameters on the diclofenac particle size' in Advanced Powder Technology, Volume 22, Issue 5, pages 587–595, September 2011, doi: 10.1016/j.apt.2010.08.010 entire document, especially section 3.1	1
A	Kayrak et al. 'Micronization of Ibuprofen by RESS' in The Journal of Supercritical Fluids, Volume 26, Issue 1, pages 17–31, May 2003, doi:10.1016/S0896-8446(02)00248-6 entire document, especially section 3.5	1
A	CN 101391156 A (ZHUJIANG HOSPITAL OF SOUTHERN [CN]) 25 March 2009 (25.03.2009) & abstract [online] EPOQUENET EPODOC & WPI & English machine translation TXPCNEA / EPO	1, 9, 12
A	WO 2013077459 A1 (UNIV TOHOKU [JP]) 30 May 2013 (30.05.2013) entire document	1
A	WO 2007072072 A2 (ACCENTUS PLC [GB]) 28 June 2007 (28.06.2007) entire document	1

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DOCUMENTS CONSIDERED TO BE RELEVANT, CONTINUED

Category*)	Bibliographic data on the document and relevant passages	Relevant to claims
A	US 5833891 A (SUBRAMANIAM BALA [US] et al.) 10 November 1998 (10.11.1998) entire document, especially figure 2	4, 11