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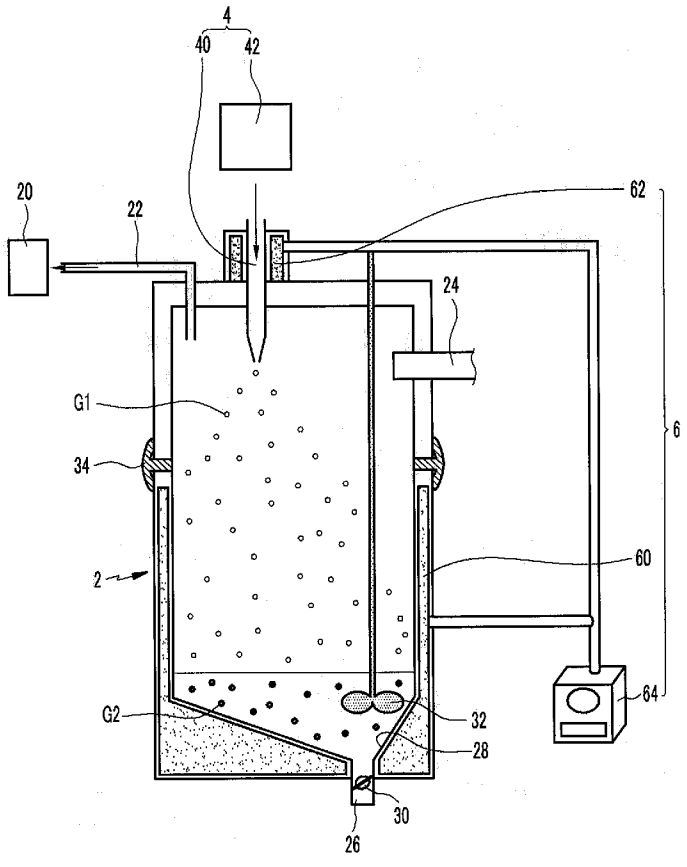
(54) Title: METHOD FOR PREPARING MICROSPHERES OF POLYMER AND MANUFACTURING APPARATUS THEROF



(57) Abstract: An apparatus and method of preparing polymer microspheres are provided. The method includes the step of injecting a solution of a biocompatible water-soluble polymer having a high molecular weight through a nozzle with the pressure ranging from 0.5bar to 15bar to form droplets, freezing the droplets by contacting the droplets with a liquefied gas to form frozen particles; removing the liquefied gas; and performing freeze-drying of the frozen particles under the temperature ranging from -70° C to -1° C and the pressure of ltorr or less.

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TITLE OF THE INVENTION

METHOD FOR PREPARING MICROSPHERES OF POLYMER AND MANUFACTURING APPARATUS THEROF

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The present invention relates to a method and apparatus for preparing polymer microspheres using a biocompatible polymer. More particularly, the present invention relates to a method for preparing polymer microspheres, comprising the steps of injecting the biocompatible polymer solution through a nozzle with a predetermined pressure to form droplets; freezing the droplets by contacting the droplets with a liquefied gas to form frozen particles; removing the liquefied gas; and performing freeze-drying of the frozen particles, to prepare the polymer microsphere. The present invention is characterized by preparing the polymer microspheres using a water-soluble biocompatible polymer under a low temperature, without using any organic solvent.

(b) Description of the Related Art

For last 30 years, there have been many researches for developing microspheres as a drug delivery system. Particularly, the microspheres have been applied to a drug which may exhibit its therapeutic effect only when administrated repeatedly, and was applied first to a drug for prostate cancer. In the case of prostate cancer, LHRH which is one of sex hormones needs to be administrated periodically in order to suppress a growing of the prostate cancer. However, a frequent administration of the LHRH is very inconvenient to patients, and thus a microsphere formulation that may be effective for a month or more by one administration was developed.

As preparing methods for the microspheres, several methods, such as a solvent evaporation drying, a phase separation, a low-temperature solvent extraction, and the like, have been well-known. However, most of them use dichloromethane or chloroform as a solvent dissolving a biodegradable polymer, which is harmful to a human body.

Y. Ogawa, *et al.* introduced a preparing method for a leuprorelin acetate depot formulation using a polymer in *Chem. Pharm. Bull.*, **36**, 2576-2581(1988). That is, the

preparation was performed by dissolving the leuprorelin acetate in an aqueous gelatin solution, and mixing the solution with a methylene chloride solution containing a polylacticglycolic acid (PLGA) to form a W/O emulsion. Sequently, the mixed emulsion was dropped into 0.25% aqueous PVA solution to form a W/O/W emulsion. Then, methylene chloride was removed and freeze-drying was performed to remove remained water. The microsphere prepared by the method of Y. Ogawa, *et al.* continuously released the leuprorelin acetate over a month with one injection. The LHRH is a peptide drug with a molecular weight of 1100, and thus, has no difficulty in being formed as microspheres. However, protein drugs with a molecular weight of 1100 or more may have a high chance to lose their specific activities by reacting with an organic solvent or a temperature variation while being produced as microspheres.

Jeffery L Cleland, *et al.*, disclosed in *Journal of controlled Release* **49** (1997), that a human growth hormone (hGH) microsphere was produced by a cryogenic process which is a improved method of a conventional W/O/W method. The hGH microsphere had slowly released the active hGH for one month after administrated into a body.

US Patent No. 5,922,253 discloses a method and apparatus related to a cryogenic process which is one of processes for preparing the hGH microspheres. In order to produce microspheres, a PLGA or a polylactic acid (PLA) is dissolved in an organic solvent, and the solution is sprayed over a region that maintains a low temperature using a liquefied gas, thereby being frozen. Then, the frozen droplets of the solution are precipitated in a solvent which may not dissolve the PLGA or the PLA in order to extract the initial organic solvent. These processes have many advantages, such as a high yield and formation of microparticles that may be used commercially. Further, these processes may be performed under a sterile state, and a size of the microparticle may be controlled. However, these processes also use the organic solvent, thereby having a chance that a protein is denaturalized. An activity of a peptide or a protein drug may be reduced due to several factors, and particularly, such activity reduction becomes great when being contacted with an organic solvent, subjected to pH variation, or exposed to a shear force.

In order to avoid problems caused by the use of organic solvents, a variety of other types of drug delivery systems have been studied, and for example, a thermosensitive polymer hydrogel has been developed. A polymer used in this formulation exhibits a sol-gel phase transition depending on temperature when dissolved

in water. This polymer is a liquid phase at a low temperature, whereby it is capable of being mixed well with a protein or a peptide drug, and becomes a solid phase by a body temperature when administrated into a body, thereby slowly releasing the protein or the peptide. In this formulation, any organic solvents that reduce an activity of the protein are not used, thereby minimizing the reduction of the activity. An exemplary polymer developed for this hydrogel system is poloxamer 407. The poloxamer 407 is a block copolymer of polyethylene glycol (PEG) and polypropylene glycol (PPG), and has a liquid phase when being dissolved in water at a low temperature and phase-changed to a solid gel phase when the temperature increases. Such polymer is called as a thermosensitive polymer, and many studies for using the polymer solution as a peptide or a protein delivery system have been continued.

However, in order that the poloxamer 407 may exhibit a controlled-release effect in a body, there is a limitation that the concentration of the polymer solution is 25% or more. Furthermore, in an actual animal experiment, most of the peptide or the protein drug was released within 1 day after an administration, thereby being unsuccessful.

US Patent No. 6,004,573 discloses a block copolymer consisting of PEG and PLGA. The copolymer has PEG-PLGA-PEG composition and exhibits a phase transition similar to that of the poloxamer 407. That is, the copolymer is in a liquid sol phase at a low temperature and phase-changed into a solid gel phase when temperature increases. The PEG-PLGA-PEG copolymer was studied as a formulation of releasing a paclitaxel, which is a water-insoluble anticancer agent, for one month. An aqueous solution of the PEG-PLGA-PEG copolymer exhibiting the sol-gel phase transition may be used as a delivery system for a protein drug, due to the properties that the aqueous solution of the copolymer is in a liquid sol phase at a low temperature, thereby being easily mixed with a protein, and phases-changed into a solid phase by a body temperature when administrated into a body, thereby releasing the protein slowly.

A polymer composition consisting of the poloxamer 407 that is biodegradable in the body is disclosed in *Controlled release society 30th annual meeting Proceedings* # 167 by X. Zaho *et al.* According to X. Zaho *et al.*, the poloxamer 407 is reacted with a disuccinimidyl carbonate (DSC) to prepare a compound wherein the poloxamer 407s are connected by degradable carbonate bonds. US Patent No. 6,348,558 discloses a

degradable polymer consisting of at least two polyalkylene oxide oligomers connected by hydrolysable carbonate bonds.

It is required to develop a method for preparing microspheres without using any organic solvents and new polymers for being applied thereto.

SUMMARY OF THE INVENTION

The object of the present invention is to provide a method for preparing microspheres using a polymer solution without using any organic solvents.

Another object of the present invention is to provide an apparatus for preparing microspheres using a polymer solution without using any organic solvents.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic view of an apparatus for preparing microspheres by a direct injection method.

FIG. 2 is a schematic view of an apparatus for preparing microspheres by an indirect injection method.

FIG. 3 is a SEM image of a prepared microsphere.

FIG. 4 is a 1H-NMR spectrum of a poloxamer disuccinate.

* Description of symbols in the drawings

2: reaction chamber

20: vacuum pump 22: vacuum tube

24: exhaust hole 26: outlet

28: inclined surface 30: valve

32: stirring blade 34: handle

4: injection unit

40: nozzle 42: pressure control pump

6: temperature control unit

60: chamber side heat exchanger 62: nozzle side heat exchanger

64: temperature control unit

G1: polymer solution

G2: spheres (frozen particles)

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to a method of preparing a microsphere comprising the steps of: injecting a biocompatible polymer solution through a nozzle with a predetermined pressure to form droplets; freezing the droplets by contacting the droplets with a liquefied gas to form frozen particles; removing the liquefied gas; and performing freeze-drying of the frozen particles, to prepare the polymer microsphere, and an apparatus for preparing the polymer microsphere.

According to the present invention, the microsphere is prepared by freezing and freeze-drying the droplets formed by injecting a biocompatible polymer solution without using an organic solvent. The biocompatible polymer is water-soluble, preferably has a high molecular weight, and more preferably exhibits a sol-gel phase transition which is a sol phase at a low temperature and becomes a gel phase at a high temperature. Particularly, it is preferable that the biocompatible polymer is a multi-block copolymer as mentioned below.

The following will describe definition of terminologies used in the present invention.

The term "biodegradable or biocompatible" means a property to be hydrolyzed or degraded by enzymes, thereby being absorbed into the body or safely eliminated from the body, after administrated into the body.

The term "microsphere" means a fine sphere having the size of 1mm or less. That is, the microsphere is a sphere-shaped fine particle, generally called as microparticle, which has the size ranging from several μm to several hundreds μm.

The term "poloxamer" is a compound wherein hydrophilic polyethylene oxide (PEO) blocks and hydrophobic polypropylene oxide (PPO) blocks are connected as the form of a PEO-PPO-PEO tri-block copolymer by ether bonds. The poloxamer has the weight average molecular weight of 1,000 to 20,000 Daltons and has hydroxyl groups at both terminuses. Particularly, a poloxamer 188 (Pluronic® F-68) and a poloxamer 407 (Pluronic® F-127) which are commercialized compounds may be used.

The term "sol-gel phase transition" means a reversible reaction that a sol phase is maintained at a specific temperature or below and becomes changed into a gel phase at specific temperature or above, or a gel phase is maintained at a specific temperature or above and becomes changed into a sol phase at the specific temperature or below. Although the specific temperature may be varied depending on the types of polymers

used, the concentration of an aqueous solution, the presence or absence of a salt, and the concentration of hydrogen ion, a range of the specific temperature may be 5 to 37°C, and preferably, 25 to 35 °C.

The term "freeze-drying" generally means a drying method by dissolving various materials in water, freezing the solution at 0 °C or below, and reducing the pressure using a vacuum pump, thereby generating sublimation of the water used as a solvent.

First, the present invention provides a method of preparing microspheres, comprising the steps of: injecting a biocompatible polymer solution through a nozzle with a predetermined pressure to form droplets; freezing the droplets by contacting the droplets with a liquefied gas to form frozen particles; removing the liquefied gas; and performing freeze-drying of the frozen particles, to prepare the polymer microsphere.

More specifically, the method of preparing the microspheres according to the present invention comprises the steps of:

i) injecting a biocompatible water-soluble polymer solution through a nozzle with a predetermined pressure to form droplets;

ii) freezing the droplets by contacting the droplets with a liquefied gas to form frozen particles;

iii) removing the liquefied gas and performing freeze-drying of the frozen particles to prepare the polymer microsphere.

Preferably, the method of preparing the microspheres according to the present invention comprises the steps of:

i) injecting a biocompatible water-soluble polymer solution having a high molecular weight through a nozzle with a pressure ranging from 0.5 to 15bar to form droplets;

ii) freezing the droplets by contacting the droplets with a liquefied gas to form frozen particles; and

iii) removing the liquefied gas and performing freeze-drying of the frozen droplets at a temperature ranging from -70 to -1°C and a pressure of 1Torr or less.

According to the present invention, the droplets may be formed by injecting a polymer solution through a nozzle with a predetermined pressure at a specific temperature or below and contacting the formed droplets with a liquefied gas, thereby forming frozen particles having the size ranging from several μm to several hundreds μm.

Thereafter, the liquefied gas is removed, and a freeze-drying of the frozen particles is performed by reducing the pressure at a low temperature, thereby preparing the polymer microspheres.

In another aspect, the present invention provides an apparatus for preparing polymer microspheres, which comprises a reaction chamber, an injection unit, and a temperature control unit.

More specifically, the apparatus according to the present invention is for preparing polymer microspheres from a biocompatible polymer solution, and comprises:

a reaction chamber connected with a vacuum pump through a vacuum tube provided with an exhaust hole through which inner gas can be exhausted and an inclined bottom surface inclined toward a discharge hole;

an injection unit including the nozzle penetrating and extending into the reaction chamber, the nozzle being connected with a pressure control pump to inject the polymer solution supplied under a predetermined pressure into the reaction chamber, thereby forming a uniform droplet; and

a temperature control unit having a chamber side heat exchanger installed at a space formed on a wall of the reaction chamber and a nozzle side heat exchanger surrounding an exposed region of the nozzle out of the reaction chamber, these heat exchanger circulating a heat exchange medium pressure-fed by the temperature control unit, thereby maintaining a temperature around the reaction chamber and the nozzle below a predetermined temperature and preventing the polymer solution from being phase-changed into a gel state.

An apparatus for preparing microspheres according to an embodiment of the present invention is shown in FIGs. 1 and 2.

An apparatus and method for preparing polymer microspheres will now be described with reference to FIGs. 1 and 2.

As shown in FIGs. 1 and 2, an apparatus for preparing a polymer microsphere comprises a reaction chamber 2, an injection unit 4 disposed at an outer side of the reaction chamber 2 and injecting a polymer solution into the reaction chamber 2, and a temperature control unit 6 maintaining an inside temperature of the reaction chamber 2 within a predetermined range.

The injection unit 4 disposed at a top-center or side of the reaction chamber 2 includes a nozzle 40 extending into the reaction chamber, and a pressure control pump

42 providing the polymer solution injected through the nozzle and forming uniform droplets in the reaction chamber 2. The reaction chamber 2 may be a 20~30L cylinder, and formed of a material enduring a vacuum state below 1torr, such as steel or stainless steel. A diameter of the nozzle 40 affects a size of the droplet, and thus may be varied according to a desired droplet size. The diameter of the nozzle 40 may be ranging from 0.1mm to 1mm. Further, a heat exchange medium may be disposed in a space defined between inner and outré surfaces of the reaction chamber 2.

A nozzle or an atomizer which is usually used in a spray-dryer may be used for the nozzle 40. Generally, the atomizer may be an external air atomizer or an internal air atomizer, and the nozzle may be a spray-dryer such as a bucci.

Further, the temperature control unit 6 includes a jacket or pipe shaped chamber side heat exchanger 60 installed at a space formed in a wall of the reaction chamber 2, and a pipe shaped nozzle side heat exchanger 62 surrounding the exposed nozzle 40 out of the reaction chamber 2. A heat exchange medium is provided and circulated to these heat exchangers 60 and 62 by a temperature control unit 64 installed separately, thereby removing a liquefied gas, maintaining a temperature around the reaction chamber 2 and the nozzle 40 at a predetermined level of -40~-5°C, and preventing the polymer solution or the droplet from being phase-changed into a gel state.

A typical refrigerating unit may be used as the temperature control unit 64, and, in this case, a CFC-based refrigerant such as HCFC22, HFC134a, R410a, or R407c may be used.

Also, a stirring blade 32 may be driven if frozen spheres that are frozen particles, are aggregated at a bottom of the reaction chamber 2 during the freezing of the droplets of the polymer solution by contacting the liquefied gas. A motor or a decelerator (not shown) may be used to rotate the stirring blade 32. The stirring blade 32 may be a propeller type, a vane type, a turbine type, a spiral type, an oar type, or an impeller type, and an embodiment of the present invention may use the propeller type stirring blade.

In addition, an upper side of the reaction chamber 2 may have a lid-structure which may be opened or closed. This may require a gasket or a packing at the lid in order to maintain a sealed state, which is for preventing an external air from inflowing inside, thereby enhancing a vacuum state of an inner space of the reaction chamber 2.

Further, when the lid-structure is added to the reaction chamber, a handle 34 may be attached on a proper place of the reaction chamber 2 in order to have easy dissembling or assembling of the lid.

An exhaust hole 24 may be disposed at the top of the reaction chamber 2 for a vaporized gas generated from the liquefied gas. After the droplets of the injected polymer solution are frozen to form frozen particles by the liquefied gas, the vaporized gas from the liquefied gas is exhausted through the exhaust hole 24 during the removing of the liquefied gas. Further, when the droplets of the polymer solution contact the liquefied gas disposed at the bottom of the reaction chamber 2, a gas generated from the liquefied gas is exhausted through the exhaust hole 24, thereby preventing an increase of an inner pressure of the reaction chamber 2 caused by a large volume of the gas generated from the liquefied gas contacting a large volume of the droplets.

There is an outlet 26 at the bottom of the reaction chamber 2 in order to collect microspheres formed by performing freeze-drying the droplets of the polymer solution. A bottom of a storage space storing the heat exchange medium may be inclined in order to collect the formed microspheres easily.

The preparing of the microspheres starts in a state where the inner space of the reaction chamber 2 is kept to a degree of vacuum below 1torr by a vacuuming operation of a vacuum pump 20 through a vacuum tube 22 and the liquefied gas is filled at the bottom of the reaction chamber 2 with a predetermined level.

A method for preparing the microspheres of the present invention comprises a step for forming the droplets by injecting the polymer solution through the nozzle 40 with the predetermined pressure.

An injection method of the polymer solution may be classified into a direct injection and an indirect injection depending on a position of an injection nozzle. Referring to FIG. 1, the direct injection has the nozzle 40 disposed at the top of the reaction chamber 2. Therefore, when the polymer solution G1 is injected, the droplets travel in the same direction as gravity, thereby rapidly contacting the liquefied gas disposed at the bottom and forming frozen spheres G2, that are frozen particles. The direct injection may quickly freeze a large volume of the aqueous polymer solution, but an aggregation of frozen droplets may happen unless agitated enough.

Referring to FIG. 2, the indirect injection has the nozzle 40 disposed at the side of the reaction chamber 2. The indirect injection injects the polymer solution under a

lower pressure than the pressure of the direct injection. Therefore, the injected polymer solution G1 travels in the horizontal direction to the nozzle 40 and in the perpendicular direction to the gravity for a while, then travels to the bottom of the reaction chamber 2 by the gravity thereby contacting the liquefied gas disposed at the bottom and forming frozen spheres G2 that are frozen particles. The indirect injection has a slower freezing process, producing a less amount of the aggregated frozen droplets, but it needs a long time to freeze the droplet.

All synthetic or natural water-soluble polymers may be used as a water-soluble polymer of the present invention. Preferably, the polymers may have a high molecular weight and more preferably, exhibit a sol-gel phase transition. The exemplary example of such polymers may be a multi-block copolymer described hereinafter. The polymer of the present invention may be selected from the group consisting of hyaluronic acid, dextran, gelatin, collagen, chitosan, methylcellulose (MC), ethylcellulose (EC), hydroxyethylcellulose (HEC), methylhydroxyethylcellulose (MHEC), hydroxymethylcellulose, hyroxypropylmethylcellulose, hyroxypropylcellulose, and a multi-block copolymer wherein tri-block copolymers are connected through a dicarboxylic linker, and each tri-block copolymer has two polyethylene oxide (PEO) blocks and a polypropylene oxide (PPO) or a polybutylene oxide (PBO) that is positioned between the two PEO blocks.

According to the method of the present invention, as molecular weight of the polymer or the concentration of the aqueous solution thereof is higher, microspheres having more densified structure may be obtained. The weight average molecular weight of the polymer may be from 25,000 to 1,000,000 Daltons, and preferably from 40,000 to 1,000,000 Daltons. When the microspheres, which are prepared from a polymer with a low molecular weight, such as poloxamer, have a problem to be dissolved before being injected into the body after reconstituted in an aqueous solution. However, microspheres prepared from a polymer with a high molecular weight have a high strength, and thus, is capable of being injected into the body with maintaining the spherical shape. Further, while a polymer with a low molecular weight may be easily broken during the freeze-drying, resulting in a low yield, the polymer with a high molecular weight as used in the present invention may retain its elasticity even after performing the freeze-drying, resulting in a low brittleness.

The polymer solution may be obtained by dissolving the water-soluble polymer into an aqueous solvent, such as distilled water, an acetate buffer solution, a phosphate buffer solution, or a peptide or a protein solution. A concentration of the polymer solution may be from 3% to 25% (w/w), preferably from 3% to 15% (w/w). When the concentration of the polymer solution is lower than the range, a particle may not be formed, and when the concentration is higher than the range, an excessively high pressure is required to spray the polymer solution.

Further preferably, the water-soluble polymer solution used in the present invention may exhibit the sol-gel phase transition, which may be applied as a good drug delivery material capable of a controlled release of a drug when administrated into the body with the drug included therein. In the present invention, the water-soluble polymer exhibiting the sol-gel phase transition may be preferably a multi-block copolymer as described hereinafter.

The water-soluble polymer solution, preferably the multi-block copolymer solution becomes a gel phase at a higher temperature than a specific sol-gel phase transition temperature due to its sol-gel phase transition property. Therefore, the injection temperature of the multi-block copolymer solution may be maintained below the sol-gel phase transition temperature. When the injection temperature is maintained below the sol-gel phase transition temperature, a viscosity of the multi-block copolymer solution is lowered, whereby the microsphere may be formed. According to the present invention, the injection temperature of the multi-block copolymer solution may be maintained 15°C or below, preferably from 5°C to 12°C. Because the polymer solution G1 exhibits the sol-gel phase transition, the temperature of the solution may also be maintained below a specific temperature by the nozzle side heat exchanger 62 that is installed around the nozzle 40, thereby preventing gelation caused by increased temperature during the injection.

According to the present invention, the polymer solution is injected through the nozzle 40 under a predetermined pressure provided from a pressure control pump 42, and a size of the droplet formed by the injection is reduced when an injection pressure increases. Therefore, the size of the droplet may be controlled by varying the injection pressure. Further, the injection speed may also be controlled by varying the injection pressure. The injection pressure may be determined considering the size of the droplet to be prepared, the injection speed, and the like. For example, the injection pressure

may be from 0.5bar to 15bar, and preferably from 1bar to 5bar. If the injection pressure is lower than the range, an injection of the aqueous solution may not occur, or the size of the droplet increases and a long time is required for the injection. If the injection pressure is higher than the range, the formed droplets may be aggregated since the injection speed is too high.

The droplets formed by injecting the polymer solution G1 fall to the bottom of the reaction chamber 2, and are changed into spheres G2, i.e., frozen particles by being contacted with the liquefied gas.

The droplets of the polymer solution injected from the nozzle 40 are rapidly frozen as soon as being contacted with the liquefied gas, which forms the frozen particles with a same size of the droplet formed by the injection. Therefore, by controlling the size of the droplet formed by the injection, the size of the frozen particle may be controlled, and the size of the droplet is controlled by varying a diameter of the injection nozzle and/or the injection pressure.

It is preferable to stir the liquefied gas in order to prevent the aggregation of the droplets during the freezing of the droplets by contacting with the liquefied gas. If the stirring speed is too high, the frozen particles G2 of the droplets may be aggregated. If the stirring speed is too low, the droplets form a chunk by adhering to each other before being frozen. Therefore, a proper stirring speed may be determined considering the kind of the polymer solution to be injected and an injection method. The preferable stirring may be from 5rpm to 300rpm, and this may be varied according to a viscosity or a concentration of the polymer solution to be injected, or manufacturing processes. More preferably, the stirring speed may be from 50rpm to 100rpm during the injection of the polymer solution, and from 60rpm to 150rpm after completion of the injecting.

In the present invention, the liquefied gas is not limited, and preferably, has a boiling point of -20 °C or below in order to freeze the droplets rapidly. More preferably, the liquefied gas may be selected from the group consisting of liquefied nitrogen, liquefied oxygen, and liquefied helium.

Further, the method of preparing the microspheres according to the present invention includes a freeze-drying process after removing the liquefied gas from the frozen droplets, i.e., the frozen particles G2. More specifically, the frozen spheres, i.e., the frozen particles G2, are freeze-dried in a freeze-drying condition formed by the chamber side heat exchanger 60, the temperature control unit 64, and the vacuum pump

20, thereby producing porous microspheres. The produced microspheres are collected through an outlet 26 after rolling along the inclined surface 28 of the bottom of the reaction chamber 2.

After the injection of the water-soluble polymer is fully completed, the temperature control unit 64 connected with the chamber side heat exchanger 60 located at the bottom of the reaction chamber 2 is driven, to increase a temperature of the bottom of the reaction chamber 2 to a level higher than the boiling point of the liquefied gas, whereby the liquefied gas is vaporized and removed. The vaporized gas is discharged through the exhaust hole 24 located at the top of the reaction chamber 2. The temperature of the bottom of the reaction chamber 2 is maintained higher than the boiling point of the liquefied gas. The temperature of the bottom of the reaction chamber 2 may be from -70 °C to -1 °C, preferably from -40 °C to -5 °C, further preferably about -20 °C.

After removing the liquefied gas, in order to performing the freeze-drying, It is preferable to maintain the temperature of the bottom of the reaction chamber 2 from -70 °C to -1 °C, preferably from -40 °C to -5 °C, and further preferably about -20 °C, and to reduce the pressure of the inner space of the reaction chamber 2 to 1torr or below by driving the vacuum pump 20.

Under the temperature and pressure condition described above, water included in the frozen particle is sublimed and dried, and thus, the porous microspheres which have many pores generated by the water sublimation and accordingly formed porous spherical structure are produced. In order to reduce the pressure of the inner space of the reaction chamber 2 by the vacuum pump 20, all exits located at the reaction chamber, such as the exhaust hole 24, the outlet 26 which collects the formed microspheres, and the injection unit 4 connected with the nozzle 40 may be sealed completely.

When the microspheres are formed by the freeze-drying of the frozen particles G2, as described above, the temperature may be about -70 to-1 °C, preferably -40 to -5 °C, and the pressure may be 1torr or less. If the temperature and the pressure of the inner space of the reaction chamber 2 are higher than the range above, the frozen particles may be aggregated or melt, which may not allow maintaining the spherical shape. The freeze-drying may be performed using the vacuum pump 20 for 6 to 12 hours, and the temperature for the initial 3hrs may be -70 to -1 °C, preferably -40 to -5 °C, more

preferably about -20 °C. Thereafter, the freeze-drying may be further performed with increasing the temperature slowly up to a room temperature.

A size of the produced microsphere may be controlled by the diameter of the nozzle 40 and the pressure generated during the injection process. The size of the produced microsphere may be 10 to 500 μm, and provided with a porous surface. The porous surface is formed by the sublimation of water contained in the polymer solution during the freeze-drying.

According to the preparing method of the present invention, a biocompatible polymer solution may include further bioactive materials. Drugs useful in the present invention may include protein or peptide drugs selected from the group consisting of various protein or peptide hormones, various antibiotics, growth hormone (GH), interferon (INF), Granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO), interleukin (IL), follicle stimulating hormone (FSH), nerve growth factor (NGP), octreotide, insulin, calcitonin, tumor necrosis factor (TNF), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), platelet derived growth factor (PDGF), bone morphogenic protein (BMP), tissue plasminogen activator (TPA), and oligonucleotide. The protein or the peptide may be contained in the buffer solution. When the biocompatible polymer may be dissolved into the buffer solution including the protein or the peptide drug(s) and then, freeze-dried, the accordingly obtained microsphere containing the protein or the peptide drug(s) may be stored for a long period since there is no water therein.

The content of the protein or the peptide drug may be 0.01 to 50 % by weight based on the weight of the polymer. The content of the protein or peptide drug may be controlled considering the releasing time, the releasing type, and the like. It is preferable that the content thereof may be 0.1 to 20 % by weight in order for the prepared microspheres to properly maintain their manufacturing features or shape.

The microspheres of the present invention may be used in the form of microspheres themselves or nanospheres. Further, the microspheres of the present invention may be applied as a controlled-released drug delivery system formulated in the form of a hybrid sol-gel depot system for drug delivery, a strip type, a bar type or a film type.

Also, in order to have additional bioactivities, the microspheres of the present invention may further include the polymer selected from the group consisting of PEG,

hyaluronic acid, dextran, gelatin, collagen, chitosan, poloxamer 407, poloxamer 188, methylcellulose, ethylcellulose (EC), hydroxyethylcellulose (HEC), methylhydroxyethylcellulose (MHEC), hydroxymethylcellulose, hydroxypropylmethylcellulose (HPMC), and hydroxypropylcellulose. The polymer may be additionally contained in the polymer solution in the amount of 0.1 to 50% by weight of based on the weight of the polymer. In this case, the microspheres of the present invention may be applied as a hybrid thermosensitive depot system, a microsphere type, a nanosphere type, a strip type, a bar type, or a film type. The gelation temperature or the gel strength of the polymer may be varied when preparing the hybrid-type drug delivery system.

When the microspheres are produced with the polymer solution including the protein or the peptide drug, various excipients and/or stabilizers for stabilizing the protein or the peptide drugs may be further added. The stabilizers and the excipients allow the protein or the peptide drugs to retain their activity without denaturing in the polymer solution, and supply a buffering effect during the freeze-drying process for preparing the microspheres, thereby maintaining a specific activity of the protein or the peptide drug.

The stabilizer may be selected from the group consisting of a phosphate buffer solution (PBS), amino acids, carbohydrates, fatty acids, and surfactants. The excipient may be selected from the group consisting of lactose, dextran, manitol, and sucrose. When the stabilizer and/or the excipient are added, amount of the stabilizer and/or the excipient may be varied according to a property of a desired microsphere. Since the physical property of the microsphere may be changed by an excessive amount of the stabilizer and/or the excipient, it is required to maintain the amount of the stabilizer and/or the excipient within a specific range, preferably 1 to 20% by weight based on the weight of the polymer.

When a metal cation is added into some proteins or peptides, the proteins or peptides may exhibit a high stability even after the freeze-drying, and control the release rate. The addition amount of the cation is varied depending on the type of the proteins or the peptides. The proteins or the peptides and the metal cation may be mixed at the mole ratio of about 1:10 to 1:5000 (mole of the protein or the peptide : mole of the metal cation). The metal cation having the effect described above may be Ca^{2+}, Zn^{2+}, or Mg^{2+}.

An administration method and dosage of the drug delivery system is determined according to a medicinal activity, a target site in the body, physiochemical characteristics, and the like. For example, the administration method may be an oral, a subcutaneous, an intravenous, or an intraperitoneal administration.

A conventional method for preparing a microsphere includes dissolving a PLGA or PLA into an organic solvent, dropping the solution into an aqueous solution, forming spherical droplets, and removing the organic solvent. However, a preparing method of the present invention includes dissolving a biocompatible polymer such as a multi-block copolymer into an aqueous solvent instead of the organic solvent, forming droplets by injecting the solution; and performing a freeze-drying using a liquefied gas. Therefore, the microspheres produced by the method of the present invention is not harmful to a human body and prevents the denaturation or deterioration of an activity of a bioactive drug such as a protein which may be included in the microspheres, thereby enhancing a stability of the drug.

Also, the controlled-release depot-type formulation may be prepared by mixing a bioactive drug such as a protein or a peptide after dissolving the biocompatible polymer of the present invention in the aqueous solvent such as water. In case the sol phase- polymer solution may be administrated by the subcutaneous injection with a syringe, therefore a temperature of the syringe storing the polymer solution needs to be maintained at a low temperature until the polymer solution is administrated. However, if it takes a long time for the administration or an external temperature is higher than 25 °C, the sol phase polymer solution stored in the syringe may be phase-changed into a solid phase, thereby making it difficult to perform the administration thereof. However, the sol-gel phase transition may be avoided until the administration when the formulation is produced in the form of the microspheres.

When the depot formulation is produced using the multi-block copolymer of the present invention, water may be included in the depot formulation, whereby hydrolysis may occur, and thus, it is difficult to be stored for a long period. Also, in the case of a freeze-storage, if the formulation is not formed in the microsphere type, the formulation becomes hardened, thereby having a difficulty in thawing. However, the microsphere formulation of the present invention does not include the water and is formed in a relatively small size, thereby capable of solving the problems.

Further preferably, the biocompatible polymer of the present invention may be a multi-block copolymer as described below.

The multi-block copolymer is an ionic polymer which is in a sol state at a low temperature and phase-changed into a gel state at a high temperature, wherein tri-block copolymers are connected through dicarboxylic linkers, and each tri-block copolymer has two PEO blocks and a PPO block or a PBO block that is positioned between the two PEO blocks.

The multi-block copolymer of the present invention forms hydrogel at a specific concentration and a specific temperature, exhibits a sol-gel phase transition, and is biodegradable. Also, the multi-block copolymer is formed by coupling through the dicarboxylic linkers, thereby increasing the molecular weight and thus, enhancing a durability of the gel. Further, since the multi-block copolymer has an ionic terminal group, the drug can be slowly released from the gel.

As an embodiment of the present invention, the multi-block copolymer may be represented by the following Chemical Formula 1.

Chemical Formula 1

M-X-O-[PEO-Y-PEO-C(=O)-R-C(=O)-O]$_n$-PEO-(PPO or PBO)-PEO-O-X-M

wherein, PEO is a polyethylene oxide block;

Y is PPO(polypropylene oxide block), PBO(polybutylene oxide block), or a combination thereof;

X is H or an anionic group;

n is an integer between 1 and 100, preferably between 3 and 100;

R is–$(CH_2)_m$- or an aryl group of -$C_{m'}$-, wherein m is an integer between 0 and 20 and m' is an integer between 6 and 12;

M is H or a cationic group preferably selected from the group consisting of Li, Na, K, Ag, Au, Ca, Mg, Zn, Fe, Cu, Co, and Ni, when X is not H; and

M does not be present when X is H.

Preferably, the polymer of the present invention may be represented as the following Chemical Formula 2.

Chemical Formula 2

M-X-O-[PEO-Y-PEO-C(=O)-R-C(=O)-O]$_n$-PEO-(PPO or PBO)-PEO-O-X-M

wherein, PEO is a polyethylene oxide block;

Y is PPO, PBO, or a combination thereof;

X is -H, -SO_3-, -PO_3^{2-}, or -C(=O)-R-C(=O)-O^-; n is an integer between 1 and 100, preferably between 3 and 100;

R is –$(CH_2)_m$- or an aryl group of -$C_{m'}$-, wherein m is an integer between 0 and 20 and m' is an integer between 6 and 2;

if X is not H, M is H or a cationic group having a univalent or divalent preferably selected from the group consisting of Li, Na, K, Ag, Au, Ca, Mg, Zn, Fe, Cu, Co, and Ni,

if X is H, M does not exist.

The PEO block of the polymer described above may consist of ethylene oxide units having a unit number ranging from 2 to 2000, preferably from 5 to 500, and more preferably from 80 to 120. In Chemical Formulas 1 and 2, the ethylene oxide unit number of each of the two PEO blocks may be same as or different from each other.

The PPO or the PBO block may have 2 to 200 units, preferably 20 to 500 units, further preferably 30 to 250 units.

According to the present invention, the multi-block copolymer includes at least 2 PEO-PPO (or PBO)-PEO units, and each unit may be such as PEO-PPO-PEO, PEO-PBO-PEO, or a PEO-(a combination of PPO and PBO)-PEO.

The multi-block copolymer of the present invention may have a weight average molecular weight of 25,000 to 1,000,000 Daltons, preferably 40,000 to 1,000,000 Daltons, more preferably 40,000 to 500,000 Daltons, and further preferably 80,000 to 120,000 Daltons.

The term "multi-block" copolymer in the present invention refers to a copolymer wherein a polyethyleneoxide block is linked to a polypropyleneoxide or polybutyleneoxide block, which is, in turn, linked to a polyethyleneoxide block and the resulting at least 2 PEO-PPO (or PBO)-PEO blocks are connected through biodegradable dicarboxylic linkers.

A ratio of the molecular weight between PEO and PPO or PBO may be varied so long as the water-soluble property of the polymer is retained, for example ranging from 0.2:1 to 40:1, preferably 1:1 to 7.5:1, and more preferably 1:1 to 5:1. The PEO

block may be contained in the amount of 10 to 85% by weight, preferably 40 to 85% by weight, based on a PEO-PPO (or PBO)-PEO unit.

The multi-block copolymer of the present invention, i.e., the PEO-PPO (or PBO)-PEO unit is connected through the dicarboxylic linker capable of being hydrolyzed in the body.

The term "dicarboxylic linker" used in the present invention refers to an alkyl or an aryl compound having 2 carboxyl groups in a molecule, such as oxalic acid, malonic acid, succinic acid, adipic acid, etc, and preferably, a nontoxic compound to the human body. Particularly, the dicarboxylic linker may be selected from the group consisting of alkyl dicarboxylic acids including malic acid, oxalic acid, succinic acid, glutaric acid, adipic acid, sebacoyl acid, suberic acid, dodecanoic acid, and the like; unsaturated dicarboxylic acids including fumaric acid, maleic acid, and the like; and aryl dicarboxylinc acids including phthalic acid, terephthalic acid, and the like.

The dicarboxylic linker may be connected with hydroxyl groups positioned at both termini of PEO-PPO (or PBO)-PEO by ester bonds, and the ester bonds may be hydrolyzed in an aqueous solution or in the body or degraded by an enzyme, whereby the multi-block copolymer is degraded to carboxylic acid and PEO-PPO (or PBO)-PEO unit.

The both terminal ends of the multi-block copolymer of the present invention are hydroxyl or ionic groups. Preferably, anionic groups of the both terminal ends may be $-SO_3-$, $-PO_3^{2-}$, $-C(=O)-R-C(=O)-O^-$, and the like. A salt corresponding to the anionic group may be a monovalent metal cation such as Li, Na, K, Ag, or Au, or a divalent cation such as Ca, Mg, Zn, Fe, Cu, Co, or Ni.

Particularly, when the polymer having anionic groups at the both terminal ends forms a complex by reacting with divalent cation, the polymer may maintain the more stable gel state, thereby capable of continuously releasing a drug therefrom. Also, when the polymer containing anionic groups is mixed with a cationic drug in an aqueous solution, an ion salt may be formed, thereby decreasing the initial burst rate of the drug from the multi-block copolymer gel, and enhancing the durability of the drug release. Further, when a divalent cationic metal salt, such as calcium chloride, zinc chloride, or magnesium chloride is added to a mixed solution of the multi-block copolymer of the present invention having anionic groups at its terminal ends and a drug having anionic group, the divalent metal cation forms a complex with the drug and the multi-block

copolymer, which allows sustained release of drugs from the gel. Therefore, the multi-block copolymer of the present invention can be effectively applied as a non-ionic and ionic drug delivery system.

In an embodiment of the present invention, the multi-block copolymer PEO-PPO (or PBO)-PEO may be a commercialized poloxamer.

The poloxamer is a tri-block copolymer having a hydrophilic PEO block and a hydrophobic PPO block that are interconnected in the PEO-PPO-PEO tri-block form by ether bonds. The poloxamer has a weight average molecular weight of 1,000 to 20,000 Daltons and has hydroxyl groups as both terminal ends. In one embodiment of the present invention, the poloxamer may be poloxamer 188 (Pluronic® F-68), or poloxamer 407 (Pluronic® F-127). In order to produce the polymer of the present invention, the poloxamer may or may not be purified, but a polymer having a high molecular weight may be produced easily when the poloxamer is purified. The purification of the poloxamer may be implemented by precipitating in hexane after dissolving the poloxamer in methylene chloride, or by a phase separation in n-propanol/H_2O solvent as disclosed in US Patent No. 5,800,711.

A method of preparing the multi-block copolymer of the present invention will be described, as follows.

More specifically, according to the present invention, the method of preparing a multi-block copolymer with terminal hydroxyl ends comprising:

1) slowly adding dicarboxylic acid dichloride to PEO-PPO (or PBO)-PEO for 6 hours or more, and allowing the reaction to proceed for 12 hours or more, wherein the addtion amount of the dicarboxylic acid dichloride is 0.5 to 1.0 equivalents based on 1 equivalent of the terminal hydroxyl end of PEO-PPO (or PBO)-PEO;

2) adding an additional 0.1 equivalents of PEO-PPO (or PBO)-PEO to the above reaction solution and allowing the reaction to proceed for 2 hours or more to generate a multi-block copolymer;

3) precipitating the produced multi-block copolymer in an ether solvent and then dissolving the precipate in methanol; and

4) slowly adding ether to the methanol solution dissolving the copolymer so that the volume ratio of methanol/ether is 1/1 to 1/20 to precipitate the copolymer.

Alternatively, the method of preparing a multi-block copolymer with terminal

carboxyl ends comprising:

1) slowly adding dicarboxylic acid dichloride to PEO-PPO (or PBO)-PEO for 6 hours or more, and allowing the reaction to proceed for 12 hours or more, wherein the addtion amount of the dicarboxylic acid dichloride is 0.5 to 1.0 equivalents based on 1 equivalent of the terminal hydroxyl end of PEO-PPO (or PBO)-PEO;

2) adding an additional 1 equivalent or more of dicarboxylic acid dichloride based on 1 equivalent of the terminal hydroxyl end of PEO-PPO (or PBO)-PEO to the above reaction solution and allowing the reaction to proceed for 2 hours or more to generate a multi-block copolymer;

3) precipitating the produced multi-block copolymer in an ether solvent and then dissolving the precipate in methanol; and

4) slowly adding ether to the methanol solution dissolving the copolymer so that the volume ratio of methanol/ether is 1/1 to 1/20 to precipitate the copolymer.

Further, the polymer of the present invention having carboxyl groups at both terminal ends may be dissolved in a solvent capable of being mixed with water, such as acetone, acetonitrile, or dioxane, and subsequently, neutralized with sodium carbonate or sodium hydrogen carbonate, thereby preparing a multi-block poloxamer having a sodium dicarboxylic acid salt at both terminal ends.

Further, the polymer having the sodium dicarboxylic acid salt at the both terminal ends may be treated with an aqueous solution of calcium chloride, zinc chloride, magnesium chloride, iron chloride, copper chloride, silver nitrate, potassium chloride, or lithium chloride, and then, a dialysis be preformed, to produce a polymer having metal carboxylate groups at both terminal ends.

A multi-block copolymer having a sulfate or phosphate groups at terminal ends may be produced by a method including:

1) dissolving a multi-block copolymer with hydroxyl groups at both terminal ends in a solvent and reacting the solution with sulfate trioxide pyridinyl complex ($C_5H_5NSO_3$) or phosphorus oxychloride ($POCl_3$); and,

2) neutralizing with sodium carbonate or sodium hydrogen carbonate.

A multi-block copolymer having other anionic groups at terminal ends may also be produced by any method well-known in the relevant art using the multi-block copolymer of the present invention.

In the above reaction, a dicarboxylic dihalide may be directly reacted as a dicarboxylic linker, and if a dicarboxylic acid is a starting material, the dicarboxylic acid may be activated by oxalic chloride which transforms the dicarboxylic acid to a dicarboxylic dichloride.

The reaction may be performed with a solvent or without any solvent, and preferably, the solvent may be selected from the group consisting of dichloromethane, chloroform, tetrahydrofuran, acetonitrile, acetone, toluene, and dioxane.

According to the present invention, the polymerization rate and degree capable of determining the average molecular weight of the polymer of the present invention may be controlled by controlling the reaction temperature and time. Although the reaction temperature may be varied depending on the boiling point of the solvent used, preferably the reaction temperature may be within the range of 60 to 120°C, and the reaction time may be within the range of 12 to 72 hours.

A catalyst, such as tin octoate, zinc chloride, and the like, may be used in order to increase the reaction rate. The reaction rate may also be increased by using an amine such as pyridine, dimethylaminopyridine, imidazole, triethylamine, and the like, in the amount of 2 equivalents based on 1 equivalent of the dicarboxylic acid. However, it is preferable not to use the catalyst or the amine in order to enhance a purity of the polymer of the present invention.

The polymer may be purified by any known method in the relevant art, and preferably by a precipitation using a solvent that dissolves the reactant but does not dissolve the product.

The polymer of Chemical Formula 1 dissolved in distilled water with the concentration of 2 to 40% forms a sol state at a temperature of 2 to 8 °C and phase-changed to a gel state at a temperature of 30 to 50 °C.

A polymer of the present invention may form a gel state at a lower concentration of 10% compared to poloxamer, thereby reducing toxicity to the body, and has a higher gelation temperature, thereby being easily injected. Further, the polymer of the present invention may increase its molecular weight by forming the multi-block of PEO-PPO (or PBO)-PEO units, thereby maintain the gel state for a long period in the body or aqueous solution. Therefore, when the polymer of the present invention is used as a drug delivery system, the drug may be continuously released for 24 hours or more with one

injection, thereby overcoming the disadvantage of the conventional poloxamer which has a short sustain period.

The multi-block copolymer of the present invention is hydrolysable due to an ester bond formed by the carboxylic linker, and the degraded PEO-PPO (or PBO)-PEO unit having a low molecular weight and dicarboxylic acid are water-soluble, thereby being easily eliminated out of the body. Since a degradation rate of the polymer is proportional to the number of the dicarboxylic linkers contained in the polymer, the degradation rate and the size of hydrolysed products may be controlled by a size and number of the blocks.

A microsphere produced with the multi-block copolymer of the present invention may be used as a drug delivery system, when the drug is included in the microsphere. The applicable drug may be any drug, for example a nonionic drug or an ionic drug, preferably an ionic drug such as a peptide or a protein containing a large number of carboxylic and amino ionic groups in the molecule. The peptide or the protein may be e growth hormone (GH), interferon (INF), granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO), interleukin (IL), follicle stimulating hormone (FSH), nerve growth factor (NGP), octreotide, insulin, calcitonin, tumor necrosis factor (TNF), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), platelet derived growth factor (PDGF), bone morphogenic protein (BMP), tissue plasminogen activator (TPA), and oligonucleotide. The protein may be a natural form or a modified form with a polymer such as polyethylene glycol Before mixed with the polymer of the present invention, some proteins may be mixed with a metal ion such as Zn^{2+}, Ca^{2+}, Cu^{2+}, Mg^{2+}, and the like, to form a complex, leading to delay the release of the drug from a gel.

The microsphere of the present invention may include the multi-block copolymer of 40 to 99.9% by weight, and the drug of 0.01 to 60% by weight. The multi-block copolymer solution with the concentration of 0.5 to 50% may be used as a drug delivery system so long as it exhibits the phase transition.

In order to produce the peptide or protein drug delivery formulation, it is necessary to prepare an aqueous solution containing the multi-block copolymer of the present invention. Since the polymer of the present invention is easily dissolved at a low temperature of about 4°C and not easily dissolved at a room temperature of about 25°C, it is preferable to dissolve the polymer at the low temperature. An amount of the

multi-block copolymer capable of being dissolved may be limited according to a molecular weight. When the molecular weight of the multi-block copolymer is about 100,000, it may be dissolved in water with the maximum concentration of 30%, preferably with the concentration of 4 to 20%. Therefore, when the peptide, a protein, or a water-soluble drug is mixed with the multi-block copolymer solution at a low temperature and then administrated by a subcutaneous or an oral route, the aqueous solution becomes a gel state at the body temperature, thereby releasing slowly the peptide, the protein, or the water-soluble drug included therein.

The present invention will now be described more fully with exemplary embodiments of the invention. The invention may, however, be embodied in many different forms and should not be construed as being limited to the embodiments set forth herein.

EXAMPLE 1: Synthesis of multi-block copolymer using succinyl dichloride as link

10g of Pluonic F-127 (BASF; poloxamer 407) was put into a 100mL round bottom flask together with a magnetic bar, and moisture contained in the Pluonic F-127 for 2 hours was removed by heated to 120°C and decompressing (1torr or less) in oil bath. The decompressing was removed, and the reaction temperature was set to 100°C with flowing nitrogen. Subsequently, 100mL of acetonitrile was added to the flask. The reaction flask was equipped with a dean stark and a cooler. 20mL of distilled acetonitrile was removed through the dean stark to remove the moisture from reactants. Then, 96uL (1 equivalent of the polymer) of succinyl dichloride was added to a reservoir in the dean stark, and reacted for 24 hours. After 24 hours, in order to substitute terminal groups of a synthesized oligomer of poloxamer 407 with carboxylic groups, 96uL of the succinyl chloride was added again to the reservoir in the dean start again and reacted for 24 hours. The synthesized oligomer of poloxamer 407 was precipitated in 1L of diethylether and filtered, to obtain the product (8.2g).

8g of the product was dissolved again in 16mL of methanol, and purified 2 times by being precipitated in diethylether and filtered. Subsequently, the product was vacuum-dried and 5.7g of poloxamer oligomer having a narrow molecular weight distribution was obtained.

The molecular weight of the poloxamer oligomer was measured by a GPC, and as the result, the weight average molecular weight was 90,700. The synthesis was confirmed by a 1H-NMR, and the 1H-NMR spectrum is shown in FIG. 4.

EXAMPLE 2: Synthesis of a poloxamer oligomer using oxalyl chloride as a linker

The same method was used as EXAMPLE 1 except using oxalic chloride as a dicarboxylic linker. The molecular weight of an obtained poloxamer oligomer was 91,300.

EXAMPLE 3: Synthesis of a poloxamer oligomer using adipoyl dichloride as a linker

The same method was used as EXAMPLE 1 except using adipoyl dichloride as a dicarboxylic linker. The molecular weight of an obtained poloxamer oligomer was 96,300.

EXAMPLE 4: Synthesis of a poloxamer oligomer using suberoyl dichloride as a linker

The same method was used as EXAMPLE 1 except using suberoyl dichloride as a dicarboxylic linker. The molecular weight of an obtained poloxamer oligomer was 97,800.

EXAMPLE 5: Synthesis of a poloxamer oligomer using sebacoyl dichloride as a linker

The same method was used as EXAMPLE 1 except using sebacoyl dichloride as a dicarboxylic linker. The molecular weight of an obtained poloxamer oligomer was 124,000.

EXAMPLE 6: Synthesis of a poloxamer oligomer using dodecandioyl dichloride as a linker

The same method was used as EXAMPLE 1 except using dodecandioyl dichloride as a dicarboxylic linker. The molecular weight of an obtained poloxamer oligomer was 104,000.

EXAMPLE 7: Synthesis of a poloxamer oligomer using terephthaloyl dichloride as a linker

The same method was used as EXAMPLE 1 except using terephthaloyl dichloride as a dicarboxylic linker. The molecular weight of an obtained poloxamer oligomer was 87,000.

EXAMPLE 8: Synthesis of a poloxamer oligomer using fumalic acid as a linker

10g of fumaric acid and 22g of oxalic chloride (2 equivalents based on 1 equivalent of fumaric acid) were quantified and added in 50mL of acetonitrile, and reacted for 6 hours at 50°C. After reaction was completed, excess oxalyl chloride was removed in vacuum condition, thereby obtaining reactive fumaroyl dichloride. The synthesized fumaroyl dichloride was used as a dicarboxylic linker, and the same method was used as EXAMPLE 1 to synthesize a poloxamer oligomer. The molecular weight of the obtained poloxamer oligomer was 85,400.

EXAMPLE 9: Synthesis of a poloxamer oligomer using maleic acid as a linker

The same method was used as EXAMPLE 8 except using maleic acid. The molecular weight of an obtained poloxamer oligomer was 82,700.

EXAMPLE 10: Synthesis of a poloxamer oligomer using malic acid as a linker

The same method was used as EXAMPLE 8 except using malic acid. The molecular weight of an obtained poloxamer oligomer was 84,000.

EXAMPLE 11: preparing a microsphere using a multi-block copolymer solution

700 mg of the multi-block copolymer prepared in EXAMPLE 1 (molecular weight: 101,500) was dissolved in 9.3g of distilled water, and the solution was left at 4°C for 2 hours forming 7% multi-block copolymer solution. The multi-block copolymer solution was injected through a 0.7mm nozzle with maintaining a

temperature at 10°C, thereby being contacted with liquid nitrogen. Subsequently, the temperature of the reaction chamber was maintained at -20°C, and freeze-drying is performed for 6 hours using a vacuum pump, to prepare a microsphere.

After completely drying, the microsphere was observed through an electron microscope, and a result is shown in FIG. 3. As shown in FIG. 3, the size of the microsphere is within a range of 10 to 200 μm.

EXAMPLE 12: preparing a microsphere using a multi-block copolymer solution mixed with an interferon

1.28mg of interferon-alpha (Sigma®) was dissolved in 10mL of distilled water, and 1.46mL of zinc acetate (1mg/mL) was added thereto so that the mole ratio between Zn and interferon would be 100:1, to form interferon-Zn complex. 840mg of the multi-block copolymer prepared in EXAMPLE 1 was added to the solution, and the solution was left at 4°C for 3 hours, thereby forming 7% interferon-contained multi-block copolymer solution. A microsphere is manufactured by the same method as EXAMPLE 11 using the interferon-multi-block copolymer solution. The size of the obtained interferon-contained multi-block copolymer microsphere is within a range of 10 to 200 μm.

EXAMPLE 13: preparing a microsphere using a multi-block copolymer solution mixed with a hyaluronic acid

700mg of the multi-block copolymer prepared in EXAMPLE 1 was dissolved in 9.3ml of distilled water, and the solution was left at 4°C for 3 hours to form 7% aqueous multi-block copolymer solution. 70mg of a hyaluronic acid (Kabi Inc Japan) was dissolved in the solution to prepare the aqueous multi-block copolymer-hyaluronic acid mixture solution. A microsphere was manufactured by the same method as EXAMPLE 11 using the aqueous multi-block copolymer- hyaluronic acid mixture solution.

EXAMPLE 13: preparing a microsphere using a multi-block copolymer solution mixed with a human growth hormone

2.07mg of human growth hormone (HGH) was dissolved in 1.66mL of distilled water. 300mg of the multi-block copolymer prepared in EXAMPLE 1 was added thereto and the aqueous solution was left at 4°C for 3 hours thereby, to form a HGH-

contained multi-block copolymer solution. A microsphere was manufactured by the same method as EXAMPLE 11 using the HGH-contained multi-block copolymer solution, and the size of the obtained HGH-contained multi-block copolymer microsphere is within a range of 10~200 μm.

WHAT IS CLAIMED IS:

1. A method of preparing polymer microspheres, comprising the steps of:

injecting a solution of a biocompatible water-soluble polymer having a high molecular weight through a nozzle with the pressure ranging from 0.5bar to 15bar to form droplets;

freezing the droplets by contacting the droplets with a liquefied gas to form frozen particles; and

removing the liquefied gas and performing freeze-drying of the frozen particles under the temperature ranging from -70°C to -1°C and the pressure of 1torr or less to prepare the polymer microspheres

2. The method of claim 1, wherein a weight average molecular weight of the polymer is ranging from 25,000 to 1,000,000.

3. The method of claim 1, wherein the injection is performed by a direct injection or an indirect injection.

4. The method of claim 1, wherein the liquefied gas is selected from the group consisting of liquefied nitrogen, liquefied oxygen, and liquefied helium.

5. The method of claim 1, the step of contacting the droplets with a liquefied gas is performed with stirring to prevent aggregation of the droplets.

6. The method of claim 1, wherein the biocompatible water-soluble polymer solution comprises 3 to 25% by weight of the biocompatible polymer dissolved in an aqueous solvent selected from the group consisting of distilled water, acetate buffer solution, phosphate buffer solution, and protein solution.

7. The method of claim 1, wherein the biocompatible polymer solution further comprises a bioactive material selected from the group consisting of growth hormone (GH), interferon (INF), Granulocyte colony stimulating factor (G-CSF), erythropoietin (EPO), interleukin (IL), follicle stimulating hormone (FSH), nerve growth

factor (NGP), octreotide, insulin, calcitonin, tumor necrosis factor (TNF), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), platelet derived growth factor (PDGF), bone morphogenic protein (BMP), tissue plasminogen activator (TPA), oligonucleotide, and a combination thereof.

8. The method of the claim 1, wherein the biocompatible polymer solution further comprises a bioactive material selected from the group consisting of a polyethylene glycol (PEG), hyaluronic acid, dextran, gelatin, collagen, chitosan, poloxamer 407, poloxamer 188, methylcellulose, ethylcellulose (EC), hydroxyethylcellulose (HEC), methylhydroxyethylcellulose (MHEC), hydroxymethylcellulose, hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose, and a combination thereof.

9. The method of the claim 1, wherein the biocompatible polymer solution further comprises one or more selected from the group consisting of:

stabilizers selected from the group consisting of a phosphate buffer solution (PBS), an amino acid, a carbohydrate, a fatty acid, a surfactant and a combination thereof; and

excipients selected from the group consisting of lactose, dextran, mannitol, sucrose, and a combination thereof,

in the amount of 1 to 20% by weight based on the weight of the polymer.

10. The method of any one of claims 1 to 9, wherein the biocompatible water-soluble polymer is selected from the group consisting of hyaluronic acid, dextran, gelatin, collagen, chitosan, methylcellulose (MC), ethylcellulose (EC), hydroxyethylcellulose (HEC), methylhydroxyethylcellulose (MHEC), hydroxymethylcellulose, hydroxypropylmethylcellulose (HPMC), hyroxypropylcellulose, and a multi-block copolymer, having a weight average molecular weight of 25,000 to 1,000,000 Daltons, wherein tri-block copolymers are connected through a dicarboxylic linker, and each tri-block copolymer has two polyethylene oxide (PEO) blocks and a polypropylene oxide (PPO) or a polybutylene oxide (PBO) that is positioned between the two PEO blocks

11. The method of any one of claims 1 to 9, wherein the biocompatible water-soluble polymer is represented by the following Chemical Formula 1:

Chemical formula 1

M-X-O-[PEO-Y-PEO-C(=O)-R-C(=O)-O]$_n$-PEO-(PPO or PBO)-PEO-O-X-M,

wherein, PEO is a polyethylene oxide block;

Y is PPO (polypropylene oxide block), PBO (polybythylene oxide block), or a combination thereof;

X is H or an anionic group;

n is an integer between 1 and 100;

R is–$(CH_2)_m$- or an aryl group of -$C_{m'}$-, wherein m is an integer between 0 and 20 and m' is an integer between 6 and 12;

M is H or a cationic group when X is not H; and M does not be present when X is H.

12. The method of claim 11, wherein the PEO-PPO-PEO is a poloxamer.

13. The method of claim 1, wherein the step of injecting the polymer solution is performed at a temperature ranging from 5°C to 12°C.

14. An apparatus for preparing microspheres using a biocompatible polymer solution comprises:

a reaction chamber connected with a vacuum pump through a vacuum tube, the reaction chamber being provided with an exhaust hole through which an inner gas is exhaust and an inclined surface inclined toward a discharge hole;

an injection unit including the nozzle penetrating and extending into the reaction chamber, which is connected with a pressure control pump and injects the polymer solution provided with a predetermined pressure into the reaction chamber thereby forming a homogeneous droplet; and

a temperature control unit having a chamber side heat exchanger covering some region of an outer wall of the reaction chamber and a nozzle side heat exchanger surrounding an exposed region of the nozzle out of the reaction chamber, the heat exchangers circulating a heat exchange medium pressure-fed by a temperature control unit, thereby maintaining temperature around the reaction chamber and the nozzle below

a predetermined temperature and preventing the polymer solution from being phase-changed into a gel state.

15. The apparatus of claim 14, wherein the injection unit is disposed at a top of the reaction chamber.

16. The apparatus of claim 14, wherein the injection unit is disposed in the side direction of the reaction chamber.

17. The apparatus of any one of claims 14 to 16, wherein the reaction chamber comprises a stirring blade disposed therein.

18. A microsphere prepared by the method of any one of claims 1 to 9, wherein the microsphere has a porous surface and a particle size of 10 to 500 μm.

19. A microsphere prepared by the method of claim 10, wherein the microsphere has a porous surface and a particle size of 10 to 500 μm.

20. A microsphere prepared by the method of claim 11, wherein the microsphere has a porous surface and a particle size of 10 to 500 μm.

FIG.1

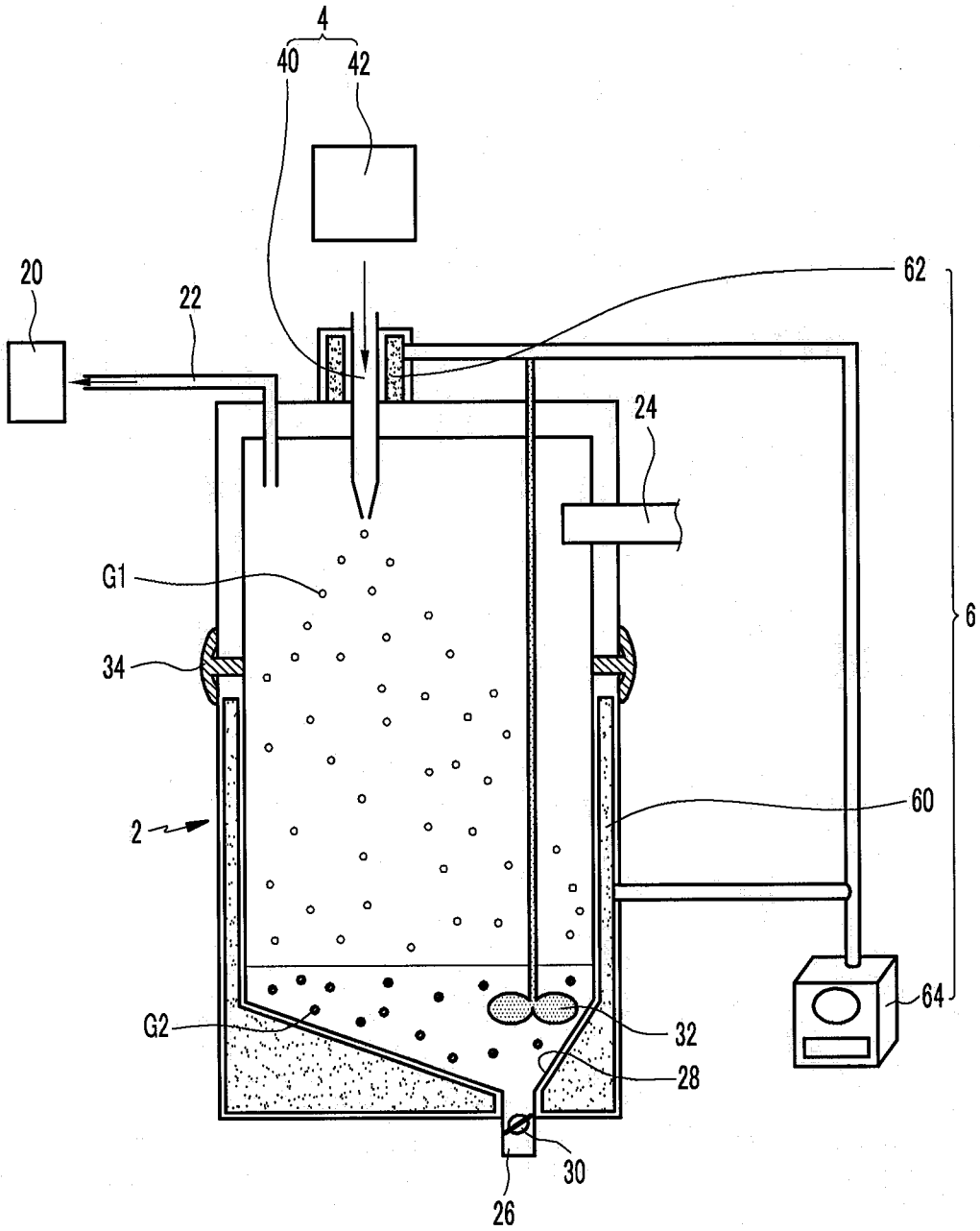


FIG.2

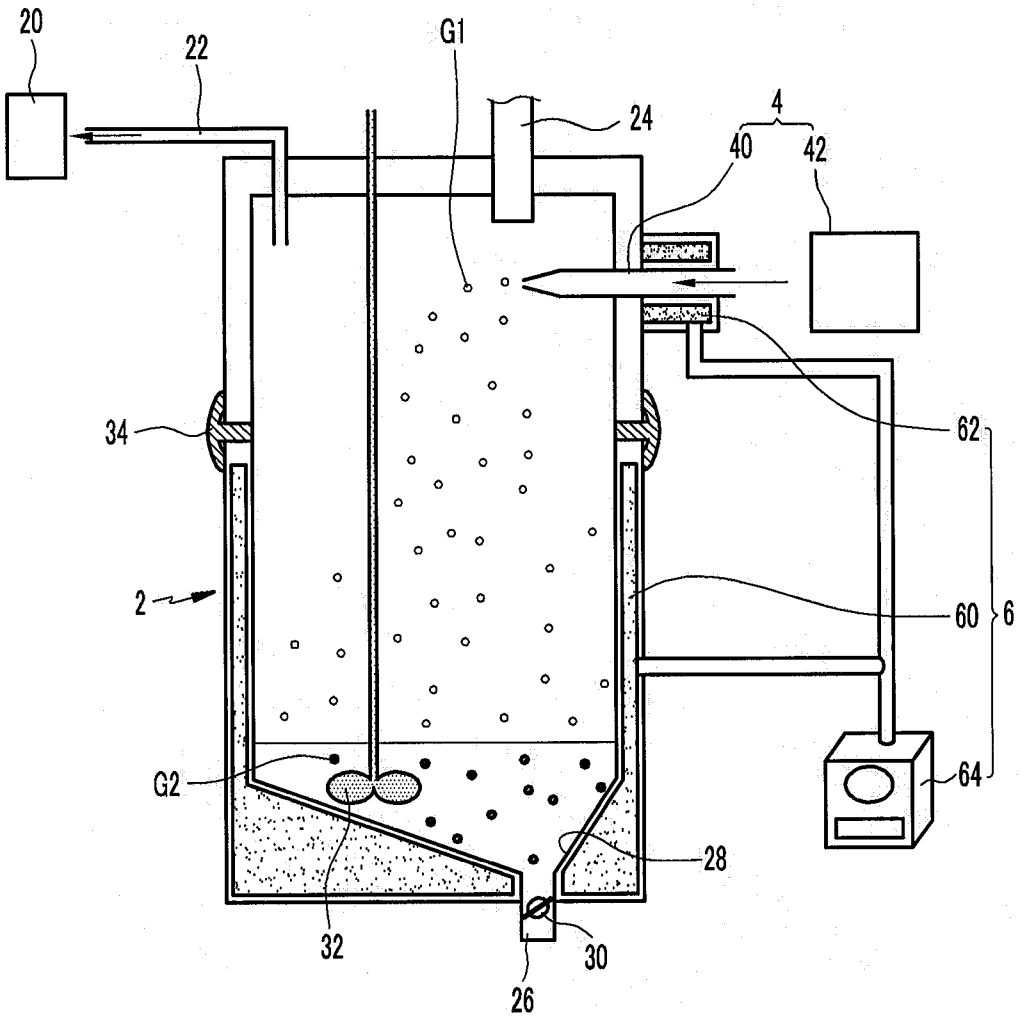


Fig. 3

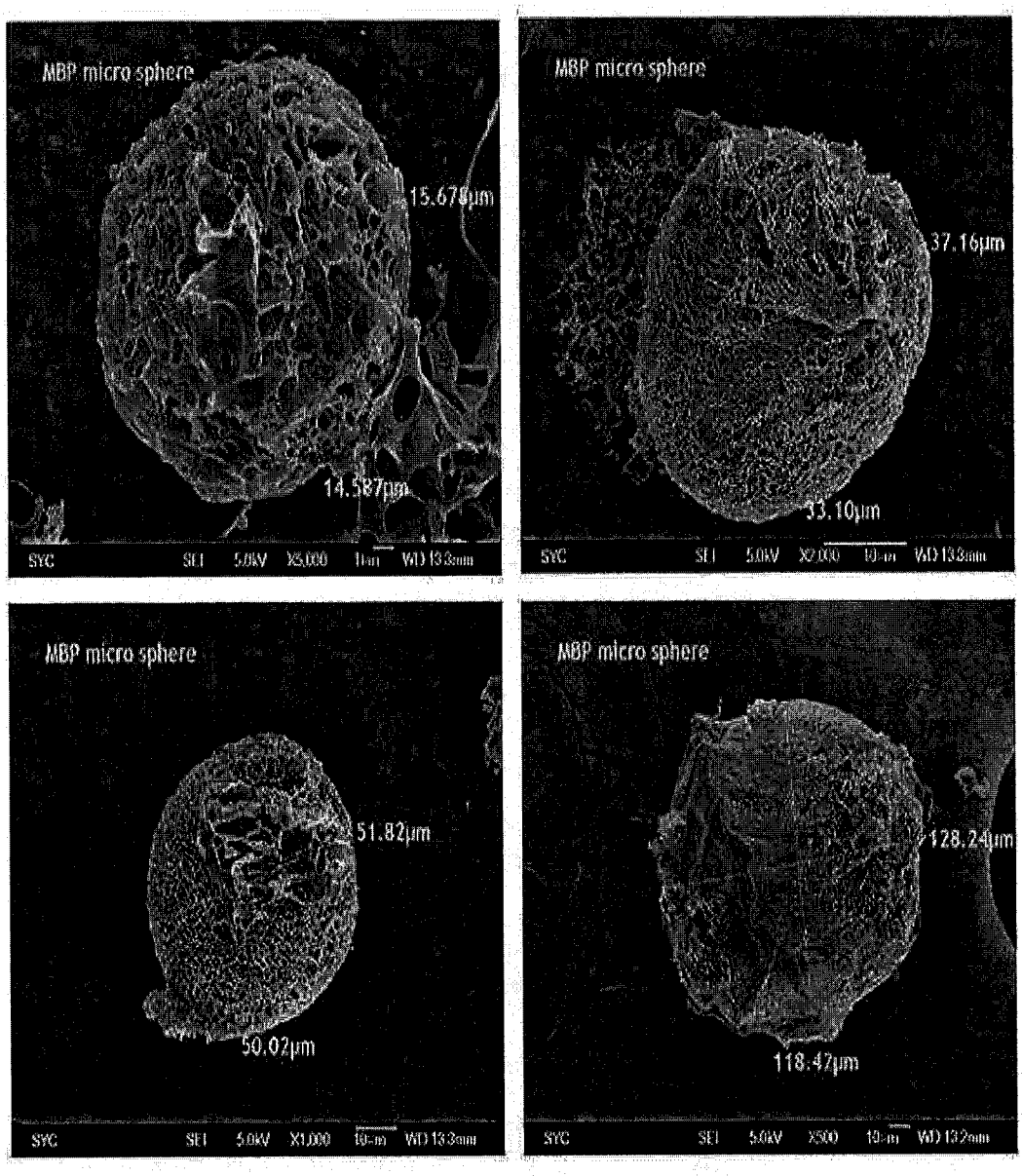
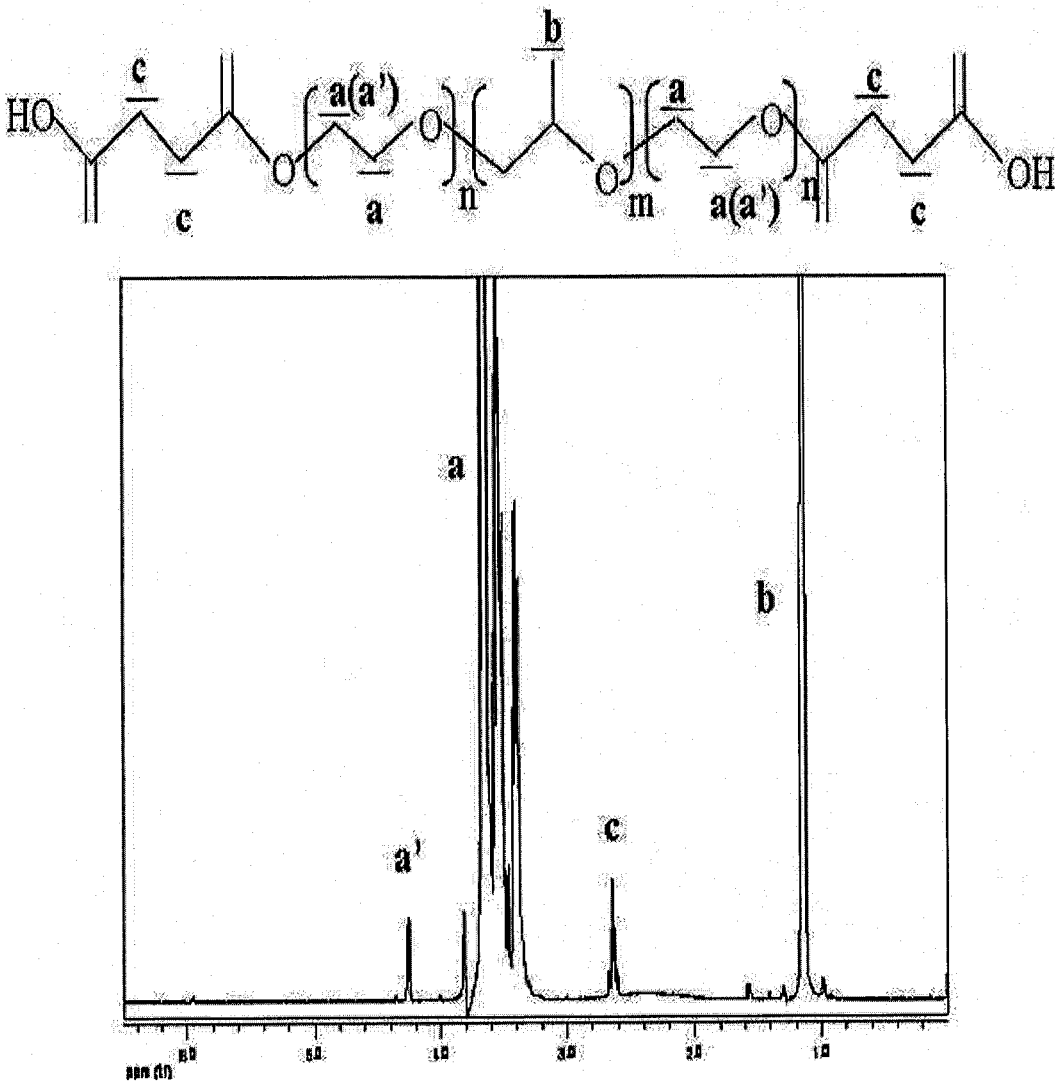

MBP micro sphere
15.678μm
14.587μm
MBP micro sphere
37.16μm
33.10μm
MBP micro sphere
51.82μm
50.02μm
MBP micro sphere
128.24μm
118.42μm

Fig. 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/004263

A. CLASSIFICATION OF SUBJECT MATTER

A61K 9/50(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC8 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean patents and applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)
eKIPASS, STN(Caplus), Pubmed
*Keywords: microsphere, spray-dry, polymer, apparatus, protein, peptide, poloxamer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	KR 10-2005-0093236 A (PACIFIC CORPORATION) 23 SEPTEMBER 2005 See pages 3-6 and claims.	1 - 13, 18 - 20
A	US 6,284,282 B1 (MAA, Y.F. et al.) 04 SEPTEMBER 2001 See the whole document.	1 - 20
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☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 JANUARY 2007 (16.01.2007)	**16 JANUARY 2007 (16.01.2007)**

Name and mailing address of the ISA/KR	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
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