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(54) Title: METHOD FOR THE PREPARATION OF POLYMERIC MICELLE VIA PHASE SEPARATION OF BLOCK COPOLYMER

(57) Abrégé/Abstract:

A method for preparing a biodegradable polymeric micellar composition using liquid polyethylene glycol as a phase separation medium comprising mixing an effective amount of a hydrophobic drug, an amphiphilic block copolymer and a phase separation medium comprising liquid poly(ethylene glycol), stirring and heating to give a solution; cooling and stirring the solution until it forms a polymeric micelle by phase separation; diluting the polymeric micelle solution in distilled water and then dialyzing it against excess water to remove the liquid polyethylene glycol; and freeze-drying the dialyzed aqueous solution to give a polymeric micellar composition in a powdered state.

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(54) Title: METHOD FOR THE PREPARATION OF POLYMERIC MICELLE VIA PHASE SEPARATION OF BLOCK COPOLYMER

(57) Abstract: A method for preparing a biodegradable polymeric micellar composition using liquid polyethylene glycol as a phase separation medium comprising mixing an effective amount of a hydrophobic drug, an amphiphilic block copolymer and a phase separation medium comprising liquid poly(ethylene glycol), stirring and heating to give a solution; cooling and stirring the solution until it forms a polymeric micelle by phase separation; diluting the polymeric micelle solution in distilled water and then dialyzing it against excess water to remove the liquid polyethylene glycol; and freeze-drying the dialyzed aqueous solution to give a polymeric micellar composition in a powdered state.

METHOD FOR THE PREPARATION OF POLYMERIC MICELLE VIA PHASE SEPARATION OF BLOCK COPOLYMER

TECHNICAL FIELD

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The present invention provides a preparation method for and the pharmaceutical application of polymeric micelles. The micelle, which is used as a carrier for hydrophobic drugs, is prepared via phase separation of a biodegradable polymeric composition containing a block copolymer having a hydrophilic poly(alkylene glycol) component and a hydrophobic biodegradable polymer component suspended in a poly(ethylene glycol) medium.

BACKGROUND ART

15 Many important drugs are hydrophobic and have limited solubility in water. In order to attain the expected therapeutic effect from such drugs, it is usually required that a solubilized form of the drug be administered to a patient. Therefore, solubilization of a poorly water soluble drug is key technology in the preparation of a formulation for oral or parenteral, especially intravenous, administration of the drug. Common methods used for 20 solubilization of poorly water soluble drugs are: i) dissolving the drug in a co-solvent of a water-miscible organic solvent and water; ii) modifying the drug to its salt that is soluble in water; iii) forming a soluble drug-complex using a complexing agent; and iv) micellizing the drug in an aqueous medium with a surfactant (Leon Lachman, "The Theory and Practice of Industrial Pharmacy", Lea & Febiger, Philadelphia, 1986).

25 Solubilization methods using surfactants, without requiring any changes in the chemical structure of a drug, have been widely used to solubilize various drugs. Non-ionic surfactants, e.g. polyoxyethylene sorbitan fatty acid esters(Tween[®]) and polyoxyethylene alkyl ethers(Brij[™] or Myrij[™]), are commonly used as the surface active agents. European Patent EP 0645145 discloses a method of solubilizing a typical poorly 30 water soluble drug, paclitaxel, by use of Cremophor EL[™], a polyoxyethylene castor oil

derivative. The use of these surfactants, however, is restricted due to toxic side effects such as hypersensitivity. They have limitations in that their poor ability to stabilize micelles can cause precipitation of the drug when the micellar solution is either stored or is to remain in place for an extended period of time.

5 Polymeric micelles have been recently investigated as potential carriers for hydrophobic drugs (Eur. J. Pharm. Biopharm. 48(1999) 101-111). Polymeric micelles are characterized by a core-shell structure consisting of hydrophobic inner core and hydrophilic outer shell. A poorly water soluble drug is entrapped within the hydrophobic core of a micelle. There are two typical methods of entrapping a poorly water soluble 10 drug in the hydrophobic core of a micelle: a) a block copolymer and a poorly water soluble drug are dissolved in water-miscible organic solvent, such as ethanol or N,N-dimethyl formamide(DMF), and the solution is dialyzed in water (Dialysis Method); and b) a drug solution of a water-immiscible organic solvent, such as dichloromethane or chloroform, is added into an aqueous polymeric solution and the organic solvent is evaporated from the 15 solution mixture (O/W Emulsion-Solvent Evaporation Method).

Yokoyama et al. (US patent Nos. 5,510,103 and 5,449,513) disclosed methods of incorporating a poorly water soluble drug into the inner core of a polymeric micelle using an A-B type diblock copolymer composed of a hydrophilic methoxypolyethylene glycol block(A) and a hydrophobic polyamino acid(B). According to the methods disclosed, an 20 aqueous micellar solution of the diblock copolymer and an organic solvent solution of the hydrophobic component are prepared in separate containers. The two solutions are then mixed and simply stirred, heated or sonicated to incorporate the hydrophobic drug into the polymeric micelles. Dialysis is then performed in which the aqueous polymer solution and the drug solution in DMF are mixed together and the mixture is dialyzed against an 25 excess of water. Therefore, these methods require preparing an aqueous micellar solution prior to incorporating a drug into the polymeric micelle [(a) G. Kwon, et al., Block copolymer micelles for drug delivery: loading and release of doxorubicin, J. Contr. Rel. 48(1997) 195~201, (b) G. Kwon, et al., Physical entrapment of Adriamycin in AB block copolymer micelles , Pharm. Res. 12(1995) 192~195].

30 X. Zhang et al. reported that a polymeric micelle prepared with a diblock

copolymer of poly(lactic acid) and monomethoxy poly(ethylene glycol) was useful as a carrier of paclitaxel (X. Zhang et al., Int. J. Pharm. 132(1996) 195-206), and Shin et al. disclose a solubilization method for indomethacin using a diblock copolymer of poly(ethylene glycol) and polycaprolactone (I. Gyun Shin et al., J. Contr. Rel., 51(1998) 13-22). In these methods, a poorly water soluble drug is incorporated in a polymeric micelle, wherein the polymers are biocompatible and biodegradable. According to their methods, a drug and a block copolymer are dissolved together in an organic solvent, especially in a water-miscible organic solvent such as tetrahydrofuran or dimethyl formamide. The polymeric micelles are prepared by first dialyzing the solution in water and then freeze-drying the aqueous micellar solution. Alternatively, a solution of a polymer and drug in a water-miscible organic solvent, acetonitrile, is prepared. The organic solvent is slowly evaporated to give a homogeneous drug-polymer matrix and the matrix is then dispersed in an aqueous medium at about 60 °C to form the polymeric micelles. It is stated that a polymeric micelle containing the drug cannot be formed if an organic solvent other than an acetonitrile, such as chloroform, dichloromethane, ethyl acetate, acetone, methanol, ethanol, or tetrahydrofuran is used for dissolving the drug and polymer. The aqueous polymeric micellar solutions are prepared by heating, ultrasonic treatment, vortex, or mechanical mixing.

As described above, a conventional solubilizing method for a poorly water soluble drug using polymeric micelles employs complicated steps including formation of an aqueous polymeric micellar solution containing a poorly water soluble drug, followed by preparation of a freeze-dried powder. Moreover, the powdered product must then be reconstituted, and when used in a hospital or other setting, it is not possible to store the product in an aqueous solution for a prolonged period because of the hydrolyzable and biodegradable component in the polymer. Another disadvantage is that this method can not be applied to a polymer having a melting temperature below about 50 °C. Furthermore, all existing methods for incorporating a drug into the micelle require using an organic solvent and preparing the polymeric micelles in an aqueous medium. It is very difficult to completely eliminate the organic solvent in the process of preparing a polymeric micelle or incorporating a drug into the micelle. In addition, the remaining

organic solvent decreases the stability of the micelle in water and makes it difficult to control the release rate of the drug.

DISCLOSURE OF THE INVENTION

The present invention discloses a preparation method of a non-aqueous polymeric micellar system without the use of significant amounts of an organic solvent having toxic side effects and require removal by evaporation. The present invention provides a method for preparing a 10 polymeric micellar composition wherein a hydrophobic drug is incorporated effectively via phase separation of a biodegradable polymeric composition containing a block copolymer having a hydrophilic poly(alkylene glycol) component and a hydrophobic biodegradable polymer component suspended in a poly(ethylene glycol) medium.

The block copolymer is mixed together with a hydrophobic drug in the liquid polyethylene glycol. A solution of the polymer and the drug is then obtained by heating the mixture. The solution is then cooled slowly and polymeric micelles having a core-shell structure form in the solution via a phase separation of the block copolymer from the 20 liquid poly(ethylene glycol). The terms poly(ethylene glycol), polyethylene glycol, or PEG, as used herein, are interchangeable and shall also be deemed to include derivatives of PEG unless otherwise specifically stated. Such derivatives will be more specifically described in the disclosure that follows. Since only the hydrophilic component block and not the hydrophobic component block of the copolymer has an affinity or attraction for the poly(ethylene glycol) matrix, the block copolymer forms a core-shell structure wherein the hydrophobic biodegradable polymer block occupies the inner core and the hydrophilic poly(alkylene glycol) block forms the outer shell in the poly(ethylene glycol) medium or 30 carrier.

In other words, the present invention concerns a method for preparing biodegradable polymeric micelles comprising the steps of:

- 1) mixing an amphiphilic block copolymer having a hydrophilic poly(alkylene glycol) A block component and hydrophobic biodegradable polymer B block component and a liquid poly(ethylene glycol) as a phase separation medium, and heating the resulting mixture to obtain a solution of said block copolymer in the liquid poly(ethylene glycol);
- 10 2) cooling the solution causing the block copolymer to separate from the poly(ethylene glycol) as polymeric micelles by phase separation in the solution;
- 3) dialyzing the solution to remove the liquid polyethylene glycol; and
- 4) freeze-drying the dialyzed aqueous solution thereby forming the biodegradable polymeric micelles in a powdered state.

Furthermore, the present invention concerns the method for preparing a biodegradable polymeric micellar composition which contains polymeric micelles and a hydrophobic drug, comprising the steps of:

- 1) mixing the hydrophobic drug, an amphiphilic block copolymer having a hydrophilic poly(alkylene glycol) A block component and hydrophobic biodegradable polymer B block component and a liquid poly(ethylene glycol) as a phase separation medium, and heating the resulting mixture to obtain a solution of the block copolymer in the liquid poly(ethylene glycol);
- 2) cooling the solution causing the block copolymer to separate from the poly(ethylene glycol) as polymeric micelles by phase separation in the solution;

3) dialyzing the solution to remove the liquid polyethylene glycol; and

freeze-drying the dialyzed aqueous solution thereby forming the polymeric micellar composition in a powdered state.

The essence of the present invention is the use of liquid polyethylene glycol as a medium for mixing and solubilization of a hydrophobic drug and the hydrophilic/hydrophobic copolymer followed by the phase separation of the polymeric micelle which provides a one step process of preparing the polymeric micelle containing a poorly water soluble drug. In contrast, conventional methods employ two steps: 1) a polymeric micelle is formed in an aqueous media and 2) a poorly water soluble drug is incorporated into the micelle in the aqueous polymer solution.

The present invention provides a method of incorporating a poorly water soluble drug into a polymeric micelle having a core-shell structure using liquid polyethylene glycol as a phase separation medium, removing the liquid polyethylene glycol and freeze-drying the resulting micellar solution.

If desired, a biocompatible water-miscible organic solvent may be added to the composition of the present invention to facilitate better solubility of a drug. The added amount of the biocompatible organic solvent depends on the solubility of the drug, and the preferred content of the biocompatible solvent is 0.1 - 20wt% based on the amount of poly(ethylene glycol) or its derivatives. The present invention is described in detail hereinafter.

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention is directed to a method for preparing a polymeric micelle having a core-shell structure using liquid polyethylene glycol as a phase separation medium and a method of incorporating a poorly water soluble drug into the polymeric micelle. A polymeric micelle composition, in a dry-state, is obtained by dialyzing the

polymeric micellar PEG solution, containing a drug or not, against water to remove the PEG followed by freeze-drying the resulting solution.

The composition containing an amphiphilic block copolymer having a hydrophilic poly(alkylene glycol) component and a hydrophobic biodegradable polymer component dispersed or suspended in a poly(ethylene glycol) medium are disclosed in copending application No WO 01/12718 A1. The amphiphilic block copolymer comprises a hydrophilic poly(alkylene glycol) component and a hydrophobic biodegradable polymer component. The polyalkylene glycol suitable for the hydrophilic component in the block copolymer of the present invention is a member selected from the group consisting of polyethylene glycol, monoalkoxy polyethylene glycol, and monoacyloxy poly-ethylene glycol, wherein the molecular weight of the polyalkylene glycol is preferably within the range of 1,000-20,000 Daltons.

The hydrophobic biodegradable polymer component of the copolymer of the present invention is a member selected from the group consisting of polylactides, polycaprolactone, copolymers of lactide and glycolide, copolymers of lactide and caprolactone, copolymers of lactide and 1,4-dioxan-2-one, polyorthoesters, polyanhydrides, polyphosphazines, poly(amino acid)s and polycarbonates. Preferably, the hydrophobic biodegradable polymer component of the copolymer of the present invention is a member selected from the group consisting of polylactide, polycaprolactone, a copolymer of lactide and glycolide, a copolymer of lactide and caprolactone, and a copolymer of lactide and 1,4-dioxan-2-one. The molecular weight of the hydrophobic biodegradable polymer component is preferably within the range of 1,000~20,000 Daltons, and more preferably within the range of 1,000~10,000 Daltons.

The amphiphilic block copolymer of the present invention may be an AB type diblock or an ABA or BAB type triblock copolymer comprising a hydrophilic

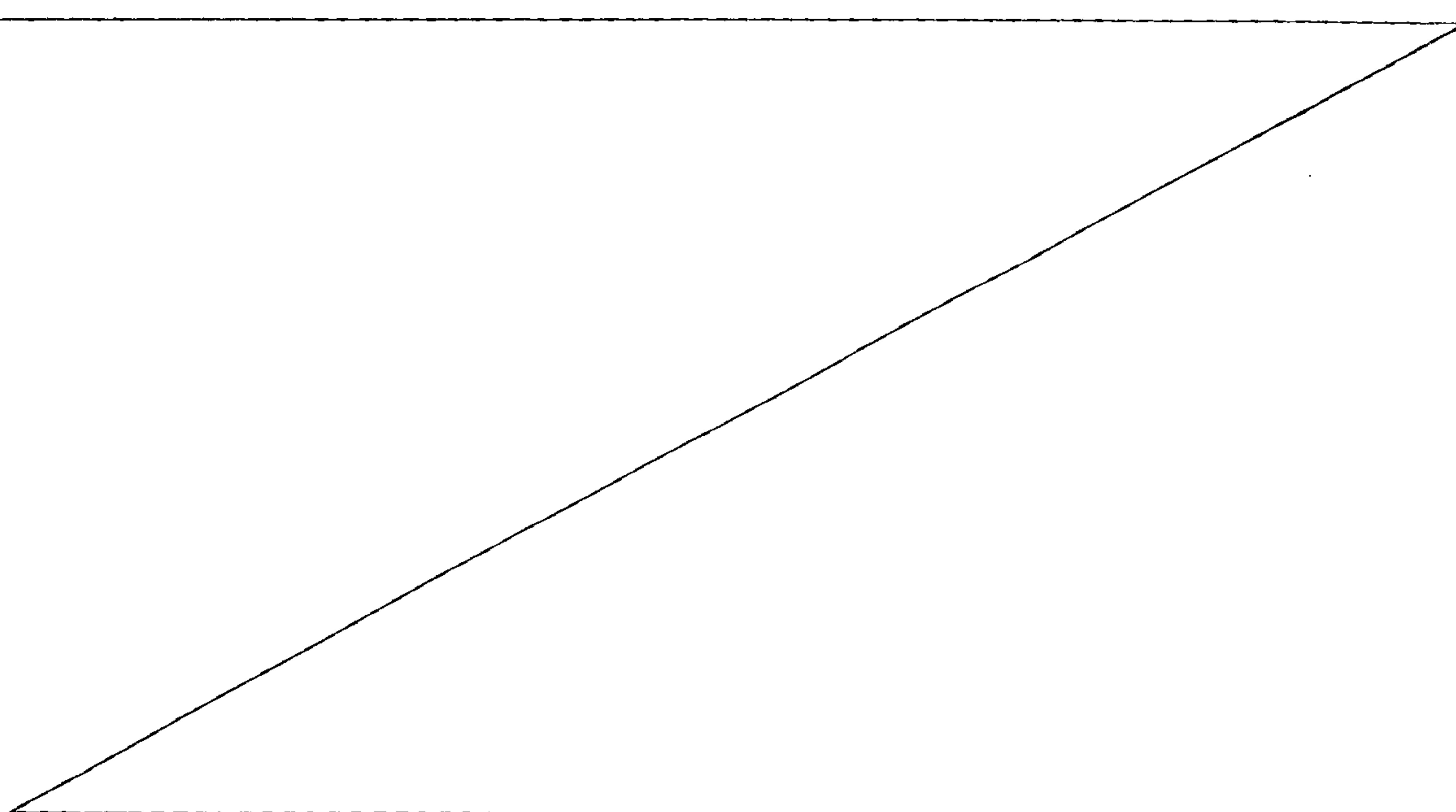
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poly(alkylene glycol) A-block component (A) and a hydrophobic biodegradable polymer B-block component(B), which forms a micelle in an aqueous medium, and is dissolved or mixed homogeneously in a poly(ethylene glycol) medium.

The amphiphilic block copolymers can be prepared according to methods described in US Patents 5,683,723 and 5,702,717. For example they may be prepared via ring opening bulk polymerization of one of the monomers, such as a lactide, caprolactone, 1,4-dioxan-2-one, or a glycolide, with a 10 polyethylene glycol derivative in the presence of stannous octoate as a catalyst. Block copolymers having a poly(amino acid) block are prepared by the reaction of an amino acid N-carboxy anhydride with a polyethylene glycol derivative. The hydrophilic polyethylene glycol block is preferably in the range of 30~70% by weight in the block copolymer, and most preferably 40~60% by weight.

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The liquid polyethylene glycol used for the phase separation medium in preparing a polymeric micelle (containing a poorly water soluble drug) of the present invention is preferably selected from the group consisting of dihydroxy, monoalkoxy, monoacyloxy, dialkoxy, or diacyloxy polyethylene glycol having a molecular weight of 200~20,000



Daltons and a melting temperature of less than 65 °C. More preferably, the liquid polyethylene glycol is selected from the group consisting of dihydroxy polyethylene glycol, dialkoxy polyethylene glycol, and diacyloxy polyethylene glycol which is a liquid at a temperature of 0~40 °C and has a molecular weight of 200~20,000 daltons, preferably 5 200~10,000 daltons, and most preferably 200~1,000 daltons. Water or an aqueous solution can be added into the liquid polyethylene glycol to facilitate the phase separation of the block copolymer micelles. Preferably the added amount will be less than 10% by weight of the liquid polyethylene glycol solution.

A small amount of an organic solvent can be added to facilitate the solubility of a 10 poorly water soluble drug in the liquid polyethylene glycol that is used for the phase separation medium. The solvent should be biocompatible and easily eliminated by evaporation or dialysis. For example, ethanol, acetic acid, or acetone can be used as the solvent, and ethanol or acetic acid is the preferred selection for this purpose. The added amount is preferably 0.1~20% and most preferably less than 10% by weight of the amount 15 of polyethylene glycol used for the phase separation medium. Such amounts of organic solvents are considered, by definition herein, to be insignificant amounts when compared to the polyethylene glycol liquid medium.

Any drug having a water solubility of less than 10 mg/ml can be used as the 20 “hydrophobic drug” or “poorly water soluble drug” to be incorporated in the polymeric micelle of the present invention. Examples of hydrophobic drugs that can be used include anticancer agents, antiinflammatory agents, antifungal agents, antiemetics, antihypertensive agents, sex hormones, and steroids. Typical examples of the hydrophobic drugs are: anticancer agents such as paclitaxel, camptothecin, doxorubicin, daunomycin, cisplatin, 5-fluorouracil, mitomycin, methotrexate, and etoposide; 25 antiinflammatory agents such as indomethacin, ibuprofen, ketoprofen, flubiprofen, dichlofenac, piroxicam, tenoxicam, naproxen, aspirin, and acetaminophen; antifungal agents such as itraconazole, and ketoconazole; sex hormones such as testosterone, estrogen, progesterone, and estradiol; steroids such as dexamethasone, prednisolone, and triamcinolone; antihypertensive agents such as captopril, ramipril, terazosin, minoxidil, 30 and parazosin; antiemetics such as ondansetron and granisetron; antifungal agents such as

amphotericin, metronidazole, and fusidic acid; cyclosporine; and biphenyl dimethyl dicarboxylic acid. The present invention is particularly useful for administering anti-cancer drugs such as paclitaxel, taxotane, doxorubicin, cisplatin, carboplatin, 5-FU, etoposide, and camptothecin; sex hormones such as testosterone, estrogen, and estradiol; 5 steroids such as triamcinolone acetonide, hydrocortisone, dexamethasone, prednisolone, and betamethasone; cyclosporine; and prostagladins.

According to a preferred embodiment of the present invention, a polymeric micelle is prepared as follows:

1) Dissolving the amphiphilic block copolymer: The amphiphilic block copolymer is added into liquid polyethylene glycol to form a mixture. The mixture is heated and/or stirred until a solution is obtained.

2) cooling and/or stirring said solution, thereby forming a polymeric micelle by phase separation from the liquid polyethylene glycol which serves as a phase separation medium.

15 3) dialyzing the polymeric micellar containing composition, formed in step (2), in liquid polyethylene glycol against excess water to remove the liquid polyethylene glycol that was used for a phase separation medium.

4) freeze-drying the dialyzed aqueous solution to give a polymeric micellar composition in a fine powder state.

20 According to the present invention, a polymeric micelle containing a hydrophobic drug is prepared by dissolving the drug together with the amphiphilic block copolymer in the liquid polyethylene glycol in step (1) as described above. At a temperature of 30~100 °C, the drug and the amphiphilic block copolymer can be easily dissolved in the liquid polyethylene glycol. If a small amount of organic solvent, such as ethanol or acetic acid, 25 is used to facilitate the solubility of a hydrophobic drug, the solution of step (1) is further stirred slowly at a temperature of 30~100 °C to evaporate the organic solvent before the solution is cooled in step (2). In any case, a drug containing polymeric micelle composition in the state of a fine powder is obtained by dialyzing the polymeric micellar solution against excess water followed by freeze-drying the resulting solution according to 30 steps (3) and (4). Before dialyzing the polymeric micellar containing composition, the

polymeric micellar containing solution can be diluted with distilled water to facilitate dialysis.

For the pharmaceutical use of the polymeric micelle prepared by the present invention, the dialyzed composition containing a poorly water soluble drug obtained in step (3) is filtered through a membrane filter, having a pore size of 0.22~0.80 μm , to sterilize the composition and then freeze-drying in an aseptic environment in step (4). When the block copolymer is dissolved in the liquid polyethylene glycol, the block copolymer content of the combined copolymer/polyethylene glycol composition is preferably 1~50% by weight, and more preferably 10~40% by weight. The poorly water soluble drug content in the polymeric micelle is preferably 0.1~20% by weight based on the total weight of the drug and the block copolymer, and most preferably 1~15% by weight. A stabilizer, such as mannitol, sorbitol, lactose, or sucrose, can be added to increase the stability of the freeze-dried micelle of the present invention. A stabilizer can be added in an amount of 0.1 ~ 200 % by weight based on the total weight of the drug and the block copolymer. The polymeric micelle prepared according to the present invention has a diameter of 10~500nm, preferably 10~20nm, and the micellar composition dispersed in saline can be used as a carrier for poorly a water soluble drug via various routes: injectable(iv, im, sc); oral; and nasal route.

While the following examples are provided for the purpose of illustrating certain aspects of the present invention, they are not to be construed as limiting the scope of the appended claims.

EXAMPLES

25 **Example 1: Preparation of mPEG-PLA diblock copolymer**

A 2g amount of monomethoxy polyethylene glycol (mPEG with a molecular weight of 2,000 Daltons) was added to a round-bottomed flask and dried at an elevated temperature of 100 °C under vacuum (0.2torr). Into the flask was added 2g of lactide and 0.02g of stannous octoate(catalyst). This mixture was stirred for 6 hours at 120 °C under nitrogen flow. The reaction product was cooled to room temperature and dissolved in

10ml of dichloromethane. The solution was then poured into cold anhydrous ether (-10~0 °C) to precipitate the polymers, namely, diblock copolymers of monomethoxy polyethylene glycol and polylactide(mPEG-PLA). The precipitated polymers were dried at 30 °C under vacuum (0.1mmHg) for 48 hours.

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Example 2: Preparation of mPEG-PLGA diblock copolymer (LA/GA=7/3)

A diblock copolymer of monomethoxy polyethylene glycol and poly(lactide-glycolide) (mPEG-PLGA) was prepared by the same method as in Example 1 using 2g of monomethoxy polyethylene glycol(molecular weight of 2,000 Daltons), 0.7g of lactide, 10 and 0.3g of glycolide in the presence of 0. 01g of stannous octoate as a catalyst.

Example 3: Preparation of mPEG-PLDO diblcok copolymer (LA/DO=5/5)

A diblock copolymer of monomethoxy polyethylene glycol and poly(lactide-p-dioxanone) (mPEG-PLDO) was prepared by the same method as in Example 1 using 2g of monomethoxy polyethylene glycol(molecular weight of 2,000 Daltons), 0.5g of lactide, 15 and 0.5g of 1,4-dioxan-2-one in the presence of 0. 01g of stannous octoate as a catalyst.

Example 4: Preparation of mPEG-PCL diblock copolymer

A diblock copolymer of monomethoxy polyethylene glycol and polycaprolactone (mPEG-PCL) was prepared by the same method as in Example 1 using 2g of monomethoxy polyethylene glycol (molecular weight of 2,000 Daltons), and 0.8g of caprolactone in the presence of 0.008g of stannous octoate as a catalyst.

Example 5: Preparation of mPEG-PLA diblock copolymer

A diblock copolymer of monomethoxy polyethylene glycol and polylactide (mPEG-PLA) was prepared by the same method as in Example 1 using 2g of monomethoxy polyethylene glycol (molecular weight of 5,000 Daltons), and 1.8g of lactide in the presence of 0. 018g of stannous octoate as a catalyst.

30

Example 6: Preparation of polymeric micelle of mPEG-PLA

(1) Formation of a polymeric micelle

A 1 g sample of the diblock copolymer prepared in Example 1(molecular weight: mPEG-PLA = 2,000-1,800 Daltons) was mixed with 4g of liquid polyethylene glycol (Mw: 600 Daltons) and stirred at 80 °C for 30 minutes to obtain a solution. The solution 5 was then slowly cooled to room temperature (25 °C) for 1 hour to obtain a composition of polymeric micelles formed in the liquid polyethylene glycol.

(2) Separation of the polymeric micelle

The composition obtained from the above step (1) was diluted with 4ml of distilled water and the aqueous solution was then put into a dialysis bag. The liquid 10 polyethylene glycol was removed from the solution by dialyzing against water for 12 hours, and an aqueous polymeric micellar solution was obtained.

(3) Sterilization and Drying

The dialyzed aqueous micellar solution obtained from the above step (2) was then filtered through a membrane filter, having a pore size of 0.22 µm, to sterilize it and then 15 freeze-dried in an aseptic environment.

Comparative Example 1: Polymeric micelle of mPEG-PLA

According to a method described in US patent No. 5,510,103, a polymeric micellar solution was prepared by dissolving 1g of the diblock copolymer prepared in 20 Example 1(molecular weight: mPEG-PLA = 2,000-1,800 Daltons) in distilled water to a concentration of 0.05%(w/v). The polymeric micellar composition in powder form was obtained by freeze-drying the aqueous micellar solution.

Each polymeric micelle prepared in Example 6 and Comparative Example 1 was dispersed in distilled water to a concentration of 0.1%(w/v) and the particle size of each 25 micellar solution was determined by a dynamic light scattering (DLS) method. The average micelle size of each preparation was nearly the same: 40nm for Example 6, and 35nm for Comparative Example 1

Example 7: Preparation of polymeric micelle of mPEG-PLA containing paclitaxel

30 (1) Formation of a polymeric micelle

A mixture was formed by adding 0.9g of the diblock copolymer prepared in Example 1(molecular weight: mPEG-PLA = 2,000-1,800 Daltons) and 0.1g of paclitaxel with 2g of liquid polyethylene glycol (Mw: 600 Daltons) and stirred at 60~90 °C for 20 minutes to give a clear solution. The solution was slowly cooled to room temperature 5 (about 25 °C) for 1 hour, and a polymeric micellar composition containing paclitaxel was obtained by phase separation from the liquid polyethylene glycol.

(2) Separation of the polymeric micelle

The composition obtained from the above step (1) was diluted with 2ml of distilled water and the aqueous solution was then put into a dialysis bag. The liquid 10 polyethylene glycol was removed from the solution by dialyzing against water for 12 hours, and an aqueous polymeric micellar solution containing paclitaxel was obtained in the dialysis bag.

(3) Sterilization and Drying

The dialyzed aqueous solution obtained from the above step (2) was filtered 15 through a membrane filter, having a pore size of 0.22 µm, to sterilize it and then freeze-dried in an aseptic environment. The average micelle size was 45nm, and paclitaxel in the micelle was 9.8% (loading efficiency = 98%) by weight based on the total weight of the drug and the block copolymer.

20 Comparative Example 2: Polymeric micelle of mPEG-PLA containing paclitaxel

A polymeric micellar composition containing paclitaxel was prepared by a method described in US patent No. 5,510,103.

Step 1: Formation of a polymeric micelle

A polymeric micellar solution was prepared by dissolving 0.9g of the diblock 25 copolymer prepared in Example 1(molecular weight: mPEG-PLA = 2,000-1,800 Daltons) in 900ml of distilled water to a concentration of 0.1%(w/v).

Step 2: Incorporation of a drug

A 0.1g of paclitaxel dissolved in 1ml acetone was added into the composition obtained from the above Step 1. The mixture was stirred at 80 °C for 2 hours and cooled 30 to room temperature (about 25 °C).

Step 3: Sterilization and Drying

The aqueous solution obtained from the above Step 2 was filtered through a membrane filter having a pore size of 0.22 μ m, to sterilize it and then freeze-dried in an aseptic environment to give a powder state of the polymeric micellar composition.

5 Each polymeric micelle prepared in Example 7 and Comparative Example 2 was dispersed in distilled water to a concentration of 0.1% (w/v) and the particle size of each micellar solution was determined by a dynamic light scattering (DLS) method. The average micelle size of each preparation was nearly the same: 55nm for Example 7, and 50nm for Comparative Example 2.

10 The amount of drug incorporated in each composition prepared in Example 7 and Comparative Example 2 was also determined by HPLC assay. The amount of paclitaxel for the composition of Example 7 was 9.8% (loading efficiency = 98%) by weight based on the total weight of the drug and the block copolymer, and 8.7% (loading efficiency = 87%) for the composition of Comparative Example 2. The polymeric micellar composition of the present invention exhibited a higher loading efficiency than that prepared according to US patent No. 5,510,103.

Example 8: Polymeric micelle of mPEG-PLDO containing cyclosporine A**(1) Formation of a polymeric micelle**

20 A mixture was prepared by adding 0.95g of the diblock copolymer prepared in Example 3 (molecular weight: mPEG-PLDO = 2,000-1,940 Daltons) and 0.05g of cyclosporine A in a solution mixture consisting of 3.2g of liquid polyethylene glycol (mw: 600 Daltons) and 0.8g of ethanol. The mixture was heated slowly to a temperature of 90 °C, with stirring, for 30 minutes to give a solution. The solution was slowly cooled to 25 room temperature (about 25 °C) for 1 hour, and a polymeric micellar composition containing cyclosporine A was obtained.

(2) Separation of the polymeric micelle

30 The composition obtained from the above step (1) was diluted with 4ml of distilled water and the aqueous solution was then put into a dialysis bag. The liquid polyethylene glycol was removed from the solution by dialyzing against water for 12 hours,

and an aqueous polymeric micellar solution containing cyclosporine A was obtained.

(3) Sterilization and Drying

The dialyzed aqueous solution obtained from the above step (2) was filtered through a membrane filter, having a pore size of 0.22 μm , to sterilize it and then freeze-dried in an aseptic environment. The average micelle size was 50nm, and cyclosporine-A in the micelle was 4.8% (loading efficiency=96%) by weight based on the total weight of the drug and the block copolymer.

10

Example 9: Polymeric micelle of mPEG-PLA containing paclitaxel

A polymeric micellar composition containing paclitaxel was prepared by the method described in Example 7 using the following ingredients:

mPEG-PLA (mw: 2,000-1,800 Daltons):	0.85g
paclitaxel:	0.15g
diethoxy polyethylene glycol (mw: 600 Daltons):	5.00g

Example 10: Polymeric micelle of mPEG-PLA containing paclitaxel

A polymeric micellar composition containing paclitaxel was prepared by the method described in Example 8 using the following ingredients:

mPEG-PLA (mw: 2,000-1,800 Daltons):	0.85g
paclitaxel:	0.15g
dimethoxy polyethylene glycol (mw: 600 Daltons):	4.00g
ethanol:	1.00g

Example 11: Polymeric micelle of mPEG-PLA containing paclitaxel

A polymeric micellar composition containing paclitaxel was prepared by the method described in Example 7 using the following ingredients:

mPEG-PLA (mw: 2,000-1,800 Daltons):	0.98g
paclitaxel:	0.02g
dimethoxy polyethylene glycol (mw: 300 Daltons):	4.00g

Example 12: Polymeric micelle of mPEG-PLA containing paclitaxel

A polymeric micellar composition containing paclitaxel was prepared by the method described in Example 7 using the following ingredients:

	mPEG-PLA (mw: 2,000-1,800 Daltons):	0.95g
5	paclitaxel:	0.05g
	diacetyloxy polyethylene glycol (mw: 300 Daltons):	4.00g

Example 13: Polymeric micelle of mPEG-PLA containing paclitaxel

A polymeric micellar composition containing paclitaxel was prepared by the 10 method described in Example 7 using the following ingredients:

	mPEG-PLA (mw: 2,000-1,800 Daltons):	0.80g
	paclitaxel:	0.10g
	polyethylene glycol (mw: 200 Daltons):	5.00g

15 Example 14: Polymeric micelle of mPEG-PLA containing cyclosporine A

A polymeric micellar composition containing cyclosporine A was prepared by the method described in Example 8 using the following ingredients:

	mPEG-PLA (mw: 2,000-1,800 Daltons):	0.90g
	cyclosporine A:	0.10g
20	dimethoxy polyethylene glycol (mw: 200 Daltons):	3.60g
	acetic acid :	0.40g

Example 15: Polymeric micelle of mPEG-PLDO containing testosterone

A polymeric micellar composition containing testosterone was prepared by the 25 method described in Example 7 using the following ingredients:

	mPEG-PLDO (mw: 2,000-1,800 Daltons):	0.95g
	testosterone :	0.05g
	polyethylene glycol (mw: 600 Daltons):	2.00g

30 Example 16: Polymeric micelle of mPEG-PLDO containing doxorubicin

A polymeric micellar composition containing doxorubicin was prepared by the method described in Example 7 using the following ingredients:

mPEG-PLDO (mw: 2,000-1,800 Daltons): 0.90g
doxorubicin : 0.10g
polyethylene glycol (mw: 600 Daltons): 2.00g

Example 17: Polymeric micelle of mPEG-PCL containing a prostaglandin

A polymeric micellar composition containing a prostaglandin was prepared by the method described in Example 8 using the following ingredients:

10	mPEG-PCL (mw: 2,000-1,800 Daltons):	0.95g
	prostaglandin :	0.05g
	polyethylene glycol (mw: 600 Daltons):	3.50g
	ethanol:	0.50g

15 The particle size and loading efficiency of drug in the polymeric micelles obtained
in Examples 7 to 17 and Comparative Example 2 are set forth in the following Table 1.

Table 1

	Drug	Content ^{a)} (wt%)	Loading efficiency ^{b)} (%)	Particle size ^{c)} (nm)
Example 7	Paclitaxel	9.8	98	45
Comparative Example 2	Paclitaxel	8.7	87	50
Example 8	Cyclosporine A	4.8	96	50
Example 9	Paclitaxel	14.1	94	50
Example 10	Paclitaxel	14.3	95	40
Example 11	Paclitaxel	1.98	99	45
Example 12	Paclitaxel	4.9	98	45
Example 13	Paclitaxel	18.8	94	50
Example 14	Cyclosporine A	9.6	96	45
Example 15	Testosterone	4.8	96	45
Example 16	Doxorubicin	9.7	97	40
Example 17	Prostaglandin	4.7	94	45

a) Content = Drug (g) / [Polymer (g) + Drug (g)] × 100

b) Loading efficiency = Loading amount (g) / Initial amount (g) \times 100

20 c) Particle size: Size of polymeric micelle containing drug

INDUSTRIAL APPLICABILITY

The above examples demonstrate that the present invention provides an efficient method to effectively incorporate a hydrophobic drug into a polymeric micelle in a polyethylene glycol separating medium. Aqueous solutions of micelles from which the polyethylene glycol has been removed can be filtered to sterilize them, freeze-dried and be stored as a stable powder formulation. Furthermore, the composition can easily be reconstituted as a solution and injected into the body and is therefore useful for the 10 intravenous administration of poorly water soluble drugs.

While the invention has been described with respect to the above specific embodiments, it should be recognized that various modifications and changes may be made to the specific embodiments described in the description by those skilled in the art which also fall within the scope of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A method for preparing biodegradable polymeric micelles comprising the steps of:

1) mixing an amphiphilic block copolymer having a hydrophilic poly(alkylene glycol) A block component and a hydrophobic biodegradable polymer B block component and a liquid poly(ethylene glycol) as a phase separation medium, and heating the resulting mixture to obtain a solution of

10 said block copolymer in said liquid poly(ethylene glycol);

2) cooling said solution causing said block copolymer to separate from said poly(ethylene glycol) as polymeric micelles by phase separation in said solution;

3) dialyzing said solution to remove the liquid polyethylene glycol; and

4) freeze-drying the dialyzed aqueous solution thereby forming the biodegradable polymeric micelles in a powdered state.

2. The method of claim 1, wherein in step 1) the amphiphilic block copolymer is present in an amount within the range of 5 to 95 wt% based on the total weight of the mixture.

3. The method of claim 1 or 2, wherein said liquid polyethylene glycol has a molecular weight of 200-10,000 Daltons and a melting temperature of less than 65°C and is selected from the group consisting of dihydroxy polyethylene glycol, dialkoxy polyethylene glycol, and diacyloxy polyethylene glycol.

4. The method of any one of claims 1 to 3, wherein said poly(ethylene glycol) phase separation medium further comprises water in an amount of 0.1-10% by weight to facilitate the phase separation.

5. A method for preparing a biodegradable polymeric micellar composition which contains polymeric micelles and hydrophobic drug, comprising the steps of:

1) mixing the hydrophobic drug, an amphiphilic block copolymer having a hydrophilic poly(alkylene glycol)

10 A block component and hydrophobic biodegradable polymer B block component and a liquid poly(ethylene glycol) as a phase separation medium, and heating the resulting mixture to obtain a solution of said block copolymer in said liquid poly(ethylene glycol);

2) cooling said solution causing said block copolymer to separate from said poly(ethylene glycol) as polymeric micelles by phase separation in said solution;

3) dialyzing said solution to remove the liquid polyethylene glycol; and

20 4) freeze-drying the dialyzed aqueous solution thereby forming the polymeric micellar composition in a powdered state.

6. The method of claim 5, wherein said liquid polyethylene glycol has a molecular weight of 200 - 10,000 Daltons and a melting temperature of less than 65°C, and is selected from the group consisting of dihydroxy polyethylene glycol, dialkoxy polyethylene glycol, and diacyloxy polyethylene glycol.

7. The method of claim 5 or 6, wherein distilled water is added into said phase separation medium in an amount of 0.1 - 10% by weight to facilitate the phase separation.

8. The method of any one of claims 5 to 7, wherein an organic solvent selected from the group consisting of ethanol, acetic acid and acetone is added into said phase separation medium in an amount of 0.1 - 20% by weight to increase the solubility of the hydrophobic drug.

10 9. The method of any one of claims 5 to 8, wherein said amphiphilic block copolymer in the mixture is in an amount of 1 - 50% by weight based on the block copolymer and phase separation medium, and said hydrophobic drug is in an amount of 0.1 - 20% by weight, based on the total weight of the drug and the block copolymer.

10. The method of any one of claims 5 to 9, wherein said hydrophobic drug is selected from the group consisting of paclitaxel, cyclosporine, prostaglandin, doxorubicin, testosterone, cisplatin, and camptothecin.

20 11. The method of any one of claims 5 to 10, wherein a stabilizing compound selected from the group consisting of mannitol, sorbitol, sucrose, and lactose is added to said mixture, with an amount of 0.1 - 200% by weight based on the total weight of the drug and the block copolymer, to increase the stability of the polymeric micelles in the powdered state.