Paclitaxel/ Steroidal Complex

Technical Field

The present invention relates to a kind of paclitaxel steroid compound and its preparation, and the present invention also relates to the use of paclitaxel steroid compound, which belongs to the technical field of pharmaceutical preparations.

Background Art

Paclitaxel (paditaxel, Taxol) has a significant anti-tumor activity, which is widely used in clinical treatment of ovarian cancer, breast cancer, non-small cell lung cancer, head cancer and neck cancer, etc. Due to its practical insoluble in water (0.006μg/ml), the common injection Taxol® intended for the clinical treatment is prepared by dissolving 30mg of paclitaxel with 5ml of mixed solution containing Cremopher EL (polyoxyethylene castor oil)/ ethanol (50:50, V/V) . However, the solvent containing a large amount of Cremopher EL in formulation releases histamine in vivo, which can cause severe allergic reaction. In order to avoiding such side effects, the desensitization is to be done as followed before the clinical use: 10mg of dexamethasone is given by oral administration 12 hours before the treatment and 10mg of dexamethasone given again by oral administraion 6 hours before the treatment, 20mg of diphenhydramine i.m., 300mg of cimetidine or 50mg of ranitidine i.v. given 30~60 minutes before the treatment. In spite of precaution, 5% to 30% of patients has different degrees of allergic

reaction in clinical practice. Moreover, the physical stability issue of paclitaxel solution is to set forth after diluting with Cremopher EL/ethanol, for example, the drug may be precipitated due to lower temperature or long infusion time, which can cause the safety risk.

As for paclitaxel injection issue, pharmacists at home and abroad have carried out excesstive study of new drug delivery system since paclitaxel marketed over 20 years, and related technical solution involves in cyclodextrin inclusion compound, liposome, polymeric micelles and nanoparticles, etc.

Cyclodextrin inclusion compound can improve the solubility of paclitaxel, but the large quantities of cyclodextrin inclusion compound lead to servere renal toxicity and producing precipitation after diluting with water, therefore it failed to the clinical stage so far.

Liposome has lots of shortcoming in terms of low encapsulation efficiency, easy leakage for the long-term storage, and producing precipitation after diluting with water, and industrialized development is struggling. Although overseas has been carried out the research for 20 years, but no product marketed. Paclitaxel liposome for injection (Li Pusu) containing 30mg of medicament per vial, its specification and dose for clinical use comply with the commercially available injection. There is no significant difference in efficacy, and the intermediate preparation process as well as desensitization pretreatment before use has been added, thus can not reflect the obvious advantages of technology.

The domestic and foreign research is extermely active in paclitaxel polymeric micelles, due to its low drug loading and unstable quality after storage, it is to should be frozen to store which limits the development of the industry. In recent years, with the emergence of novel polymer materials, polymeric micelles technology has been developed rapidly, but the new structure of the polymer material into the body, its clinical safety of drug needs to be further investigated.

Paclitaxel nanoparticles combined with albumin for injection (Code No. ABI-007) in American Bioscience Company approved by FDA in 2005 is by far the most influential paclitaxel preparation protected by the international patent and its technical solution is prepared as the following steps: using human serum albumin as drug carrier, produce paclitaxel into nanoparticles combined with albumin, sterilize, filter and freeze to drying, obtain paclitaxel injection containning 100mg of paclitaxel and 900mg of albumin per vial. Compared with the common injection, paclitaxel nanoparticles combined with albumin has two advantages, 1) there is no Cremopher EL in the formulation which eliminates the allergic reaction completely and becomes the only paclitaxel preparation for direct use without desensitization treatment in the international; 2) due to toxicity reduction and tolerability improvement, the dose amount administered by patient increases from 135 ~ 175mg/m^2 to 260mg/m^2, the crative effect is superior to that of the common paclitaxel injection. Albumin as the carrier with the characteristics of large amounts and expensive cost (the price up to 6200 RMB per vial), as well as the complex of

the intermediate procedure, limits the clinical application of paclitaxel nanoparticles combined with albumin.

For oil-in-water submicron emulsion, taking advantage of drug lipophilicity, dissolve the drug with oil phase, using the natural phospholipids as the emulsifier, high-pressure homogenizer and emulsify to produce the emulsion with average particle size less than 600nm. Due to the drug included in the internal oil phase without contact with water and air, it is possible to overcome the shortcomings of insoluble drug with low solubility and poor stability if produced the liquid preparations. Compared with the technology of liposome, submicron emulsion has more advantages of industrialized development; compared with nanoparticle combined with albumin, submicron emulsion with low manufacturing cost, can be sterilized at the terminal and directly used for infusion in clinical treatment without producing precipitation, and is also convenient and safe to use. Therefore, using submicron emulsion as a carrier to develop paclitaxel preparations has bright prospect. Although lots of trials have been carried out by the domestic and overseas researchers and the results have been shown that due to paclitaxel with practical insoluble in water and little solubility in oil (about 0.25mg/ml), if submicron emulsion is prepared directly, the drug loading is less than 0.02 mg/ml, and during the process of sterilization and storage, drug can easily transfer from oil phase to water phase, resulting in demulsification, layered or assembly. Limited by the low solubility of paclitaxel in oil, it is has not been developed so far in the international

paclitaxel submicron emulsion with high drug loading, sterilization resistance to heat and pressure as well as stable quality in long-term storage.

In order to improve the solubility of the drug in the oil phase, and breakthrough the limit of physical and chemical properties in paclitaxel submicron emulsion study, we carry out the research on "palitaxel lipid compound" and "paclitaxel submicron emuliosn prepared with the lipid compound as the intermediate carrier" in the early stage, and apply two patents, "paclitaxel lipid compound" with application number of 200810168213.X and "paclitaxel submicron emuliosn prepared with the lipid compound as the intermediate carrier" with application number of 200810168212.5.

With respect to paclitaxel lipid compound as disclosed in Patent 200810168213.X, the preferred lipid materials is the natural egg yolk lecithin, soybean lecithin and cholesterol, the weight ratio of paclitaxel and lipid materials is 1:1~19, that is amount of lipid materials is 1~19 times higher than that of paclitaxel (using phospholipids as lipid materials, the molar ratio of paclitaxel and lipid materials is 1:2.2~20; using cholesterol as lipid materials, the molar ratio of paclitaxel and lipid materials is 1:2.2~42; using cholic acid as lipid materials, the molar ratio of paclitaxel and lipid materials is 1:2.1~40.) The submicron emulsion as disclosed in Patent 200810168212.5 is prepared with paclitaxel lipid compound disclosed in Patent 200810168213.X as the intermediate carrier.

The purpose of preparing paclitaxle lipid compound lies in improving the solubility of paclitaxel in oil, which provides with a good intermediate carrier for

the follow-up preparation of submicron emulsion. But the further studies have been shown that the technical solution as claimed in Patent 200810168213.X and 200810168212.5 has the following shortcomings:

1. Phospholipids as lipid materials to producing the compound, provided that it has improved the solubility of drug in oil, maximum to 2mg/ml, the higher amount of phospholipids used can not constantly improve the solubility in oil; as the intermediate carrier to producing submicron emulsion, phospholipids with maximum drug loading of 0.5mg/ml, is unstable if stored for 6 months, which can not meet the clinical treatment.

2. Cholesterol as lipid materials to producing the compound is superior to phospholipids in improving the solubility of drug in oil, however cholesterol belongs to steroid, amount of cholesterol is 1-19 times than that of paclitaxel, which has lots of drawbacks: **(1) excessive intakes:** the intake of cholesterol for healthy adults is about 300mg~500mg (equivalent to the amount of cholesterol in 1~2 egg yolks), and the dose of paclitaxel for clinical use once is about 300mg. Compared with cholesterol and its preparations as claimed in Patent 200810168213.X, the cholesterol intake is about 300mg~5700mg with maximum amount equivalent to 19 egg yolks, and is significantly high with safety risk; **(2) instability of submicron emulsion in the long-term storage:** using cholesterol as the intermediate carrier to producing submicron emulsion, according to the dose for the clinical treatment and the specific concentration of paclitaxel, the higher amount of lipid materials in the compound, the higher total amount of the

compound encapsulated in the oil phase. Limited by the droplets volume and oil-water interface in the internal oil phase, when the total amount of the compound exceeds the contents of oil phase and oil-water interface, it is possible for parts of drug to make free to the external water phase, which leads to the low encapsulation efficiency and instability of submicron emulsion. Through the study of the submicron emulsion as claimed in Patent 200810168212.5, its encapsulation efficiency is less than 85%, and the quality of submicron emulsion is stable if stored at 4 □for 6 months, but the apparent layered has been shown if stored for 12 months, which indicates that the labelled amount has been decreased and the amount of impurity in paclitaxel has been increased; **(3) high cost of preparation:** the high amount of lipid materials and solvent during the process as well as the long time to remove the solvent lead to high cost of manufacture, which does not comply with the principles of pharmacoeconomics.

Therefore, on the basis of the two Patents above, the application carries out lots of test research, selects and optimizes the lipid materials and its amount further, in order to provide the compound with fewer quantities of lipid materials and lower cost, which has improved the safety, effectiveness and quality control in clinical practice. The inventors of the present invention have found that in a large number of lipid materials, steroid is the best lipid materials, characterized in that steroid lipid materials has strong capacity to compound with paciltaxel and using fewer quantities of steroid can increase the solubility of drug in oil to the maximum.

Using steroid compounds described in present invention as the intermediate carrier to producing oil-in-water submicron emulsion, because of the fewer total amounts of the compound encapsulated, higher encapsulation efficiency as well as not easily demulsifciation in the long-term storage, its physical and chemical stability are superior to those of submicron emulsion as claimed in Patent 200810168212.5. Paclitaxel steroid compound claimed in the present invention has laid a solid foundation for the quality control and effectiveness as well as safety in clinical practice of the follow-up submicron emulsion.

Contents of the invention

An object of the present invention is to provide with one kind of paclitaxel / steroid compound composed of the paclitaxel and steroid lipid materials. The molar ratio of paclitaxel and steroid stands 1:0.2~4, preferably 1:0.25~2, more preferably 1:0.33~1.

Paclitaxel/steroid compound set forth in the invention, wherein the steroid lipid materials belong to the natural steroidal substance or one kind of its derivatives at least; the natural steroid as claimed above can be chosen from cholesterol, 7 – dehydrocholesterol (also known as 7 - hydrogenated cholesterol), lanosterol, sitosterol, stigmasterol, sitosterolum, ergosterol, brassicasterol, mycosterol or oysters steroid; and its derivative can be chosen from cholic acid, deoxycholic acid and anthropodesoxycholic acid. The preferred steroid is

cholesterol, 7- dehydrogenation cholesterol or ergosterol, the more preferred cholesterol or ergosterol.

The antioxidant stabilizer should be used in paclitaxel/ steroid compound as cliamed in the present invention. The preferred antioxidant stabilizer can be chosen from one kind of sodium bisulfite, sodium metabisulfite, vitamin C, EDTA and its salts, vitamin E and its derivatives at least.

Another object of the instant invention lies in providing with the preparation of paclitaxel/ steroid compound, which can be prepared in accordance with the following Method 1 or 2.

Method 1 involves the following steps:

a. Mix paclitaxel and steroid in proportion, dissolve with an appropriate amount of organic solvent, and then add the antioxidant stabilizer randomly;

b. Stir under the suitable temperature condition, remove the organic solvent, vacuum drying.

Method 2 includes the following steps:

a. Dissolve paclitaxel and steroid in an appropriate amount of different organic solvents, mix, add the antioxidant stabilizer randomly;

b. Stir under the suitable temperature condition, remove the organic solvent by rotary evaporation or spray drying, vacuum drying.

As described in the preparation of the present invention, the organic solvents should be chosen from one king or several of dichloromethane, ethanol, methanol,

benzyl alcohol, acetone, ethyl acetate, tetrahydrofuran and tert-butanol; preferably one kind or several of ethanol, acetone, ethyl acetate and tetrahydrofuran. If several of organic solvents used, it refers to a mixture of organic solvents.

In the preparation of the invention, the removal of organic solvents can be obtained by rotary evaporation or spray drying.

In the preparation of the instant invention, "mix in proportion" refers to mix paclitaxel and steroid as claimed above in the application, that is the molar ratio of paclitaxel and steroid stands 1:0.2~4, preferably 1:0.25~2, more preferably 1:0.33~1; "an appropriate amount" in "an appropriate amount of organic solvents" refers to the amount of mixture for dissolving paclitaxel and steroid determined by those skilled in the art according to the conventional techniques. For example, the concentration of paclitaxel steroid compound in the solution is 0.5~16mg/ml, calculated as paclitaxel, preferably 1.0 ~ 8.0mg/ml; "the suitable temperature condition" refers to 25 □-70 □, preferably 35-55□, such as 25□, 35 □, 45 □, 55 □ or 70 □.

In the preparation of the instant invention, the stirring and vacuum drying time are subject to those skilled in the art in accordance with the conventional technique, for instance stirring time can be 0.5-3.0 hours, e.g. 0.5 hour, 1.0 hour, 1.5 hours or 2.0 hours, and vacuum drying time can be 8-48 hours, e.g. 8 hours, 12 hours, 16 hours or 24 hours.

In the preparation of the instant invention, an appropriate amount of

antioxidant stabilizer should be considered to add, and its dose complies with the commonly used in the preparation of liposome compounds in the field, generally not more than 1% of the sum of paclitaxel and cholesterol (weight).

The invention as described herein also provides with the preparation of oil-in-water submicron emulsion, dry emulsion, self-microemulsifying system or oral preparations. Wherein oil-in-water submicron emulsion or dry emulsion is obtained by dissolving paclitaxel/ steroid compound in the oil phase, which can be administered by injection intended for cancer treatment. The preparation has the advantages of high drug loading, good stability as well as no Cremopher EL in formualtion, and its safety is better than the commercial injection. Self-microemulsifying system can be obtained by dissolving paclitaxel/ steroid compound in the oil phase, then adding an appropriate amount of surfactants (emulsifier) and cosurfactant (assisting emulsifying agent) used for cancer treatment through injection, mucosal or oral administration. Oral preparations, in particular solid dosage forms such as capsules or tablets, should be obtained by adding excipients for pharmaceutical use in paclitaxel steroid compound, intended for cancer therapy through oral administration, which has higher bioavailability.

Paclitaxel/ steroid compound as claimed in the invention can also be used in preparation of anti-cancer drugs, which have been widelyt used for the treatment of ovarian and breast cancer, non-small cell lung cancer, head and neck cancer and also for gastric or pancreatic cancer.

Unless specified stated, the meanings of the science and terminology and titles

as clailmed in the invention comply with the general understanding those skilled in the filed; and unless specified pointed out, the substance to be used and its content or proportion, device, instrument, preparation conditions, etc. are well known by those skilled in the art or described in the invention.

Paclitaxel/ steroid compound as claimed in the invention has the following advantages in particular:

1) Less lipid materials used and low preparation cost: the fully compounded of drug and lipid materials is a prerequistite for improvement of drug solubility in oil. Compared with paclitaxel compound known in this field, including paclitaxel compound as claimed in Patent 200810168213.X, the molar ratio of drug and steroid lipid materials stands 1: 0.2~4, preferably 1:0.25~2, more preferably 1:0.33~1.

The molecular weight of steroid lipid materials as claimed in the present invention is 384.6~414.7, thus the molar ratio of drug and steroid is 1: 0.2, the corresponding weight ratio is 1: 0.09~1: 0.097; whereas the molar ratio is 1:4, the corresponding weight ratio is 1:1.80~1.94, that is the molar ratio of drug and steroid stands 1:0.2~4 in the preferred range, the corresponding weight ratio is 1:0.09~1.94. Accordingly the molar ratio of drug and steroid stands 1:0.25~2 in the preferred range, the corresponding weight ratio is 1: 0.11~0.97; whereas the molar ratio of drug and steroid stands more preferably 1:0.33~1, the corresponding

weight ratio is 1: 0.15~0.49.

Compared with the contents as disclosed in Patent 200810168213.X, the steroid chosen in the present invention is lipid materials, the weight ratio of drug and lipid materials reduces from 1:1~19 to 1: 0.09~1.94 (preferably 1:0.11~0.97), which decreases the amount of lipid materials, narrows the usage amount, improves the loading of paclitaxel in the compound (increased from 5%~50% to 35%~91.7%), and ensures paclitaxel to be fully compounded so that the maximum solubility of paclitaxel can be obtained in oil, which can meet the follow-up preparation of submicron emulsion. Increasing the amount of lipid materials can not continually improve the solubility of the drug in oil.

2) Less lipid materials intake: take 300mg of paclitaxel once as calculated, steroid intake should be controlled at 27mg~580mg once, preferably 33mg~290mg. Compared with 300mg~5700mg as claimed in Patent 200810168213.X, steroid intake has obviously decreased which can reduce the high risk of safety.

3) Improving the encapsulation efficiency and stability of submicron emulsion: The maximum amount of steroid in the compound of the present invention is only 1.94 times the amount of paclitaxel, preferably 0.97 times. If dissolving the compound with vegetable oil to preparing oil-in-water submicron emulsion, the less total quantities of compound within the oil phase can improve the encapsulation efficiency and physical and chemical stability in long-term storage. The comparative research has been proved that using the compound as claimed in the present invention as the intermediate carrier to preparing submicron

emulsion, the encapsulation efficiency can be kept at 90% above and the quality stability can be achieved at 4 ° C for 12 months, whereas the encapsulation efficiency of the submicron emulsion as claimed in Patent 200810168212.5 kept at 65% -85% and the apparent layered had been shown if stored at 4 ° C for 12 months and the quantity of impurity had been increased greatly.

4) Enchancing the safety: Compared with the commercially available injection, the formualtion of submicron emulsion prepared with paclitaxel/ steroid compound as the intermediate carrier excludes Cremopher EL which can avoid a severe allergic reaction triggered by Cremopher EL, reducing animal toxicity and increasing the tolerated dose.

Although the present invention has been fully described in connection with the description of figures and embodiments, it is to be noted that the invention included but not limited to these embodiments as well as the preparation method will become apparent to those skilled in the art. Moreover, according to the description, technicians in the field can equally substitute, combine, change or modify the compound as being included within the scope of the present invention.

Description of figures

Figure 1: DSC curve of the compound with different molar ratio and the physical mixture in Test Case 3

Figure 2: X -ray diffraction diagram in Test Case 6

Figure 3: Infrared spectrum in Test Case 7

Figure 4: Figure 4-1: Ultraviolet spectrum in Test Case 8

Figure 4-2: Comparison of HPLC chromatographic peaks in Test Case 8 (peak 1: paclitaxel)

Mode of Carrying out the Invention

Preparation of embodiments

Embodiment 1 Paclitaxel cholesterol compound

Take 9.0g of paclitaxel and 0.81g of cholesterol in a rotary evaporator, dissolve with 3000ml of acetone, mix at 40 ° C for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 40 ℃ for 12 hours.

Embodiment 2 Paclitaxel cholesterol compound

Take 9.0g of paclitaxel and 0.99g of cholesterol in a rotary evaporator, dissolve with 3000ml of tetrahydrofuran, mix at 25 ℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 25 ℃ for 12 hours.

Embodiment 3 Paclitaxel cholesterol compound

Take 8.5g of paclitaxel and 1.275g of cholesterol in a triangle flask, dissolve with 3000ml of tetrahydrofuran, mix at 45℃ for 1.5 hours, then remove the solvent by rotary evaporation method, vacuum drying at 45 ℃ for 16 hours.

Embodiment 4 Paclitaxel cholesterol compound

Take 8.0g of paclitaxel and 1.84g of cholesterol in a triangle flask, dissolve with 3000ml of acetone, mix at 35℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 35 ℃ for 10 hours.

Embodiment 5 Paclitaxel cholesterol compound

Take 7.0g of paclitaxel and 3.15g of cholesterol in a triangle flask, dissolve with 3000ml of acetone, mix at 50℃ for 2 hours, then remove the solvent by rotary evaporation method, vacuum drying at 50 ℃ for 15 hours.

Embodiment 6 Paclitaxel cholesterol compound

Take 7.0g of paclitaxel and 4.2g of cholesterol in a triangle flask, dissolve with 5000ml of ethyl acetate, mix at 55℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 40 ℃ for 15 hours.

Embodiment 7 Paclitaxel cholesterol compound

Take 5.2g of paclitaxel and 4.73g of cholesterol in a rotary evaporator, dissolve with 3000ml of dichloromethane, mix at 60℃ for 2.5 hours, then remove the solvent by rotary evaporation method, vacuum drying at 55℃ for 15 hours.

Embodiment 8 Paclitaxel cholesterol compound

Take 5.2g of paclitaxel, 4.85g of cholesterol and 0.05g of vitamin E in a rotary evaporator, dissolve with 4000ml of tetrahydrofuran, mix at 40℃ for 1 hour, then remove the solvent by spray drying for 15 hours, vacuum drying at 55℃ for 15 hours.

Embodiment 9 Paclitaxel cholesterol compound

Take 5.1g of paclitaxel and 4.95g of cholesterol in a rotary evaporator, dissolve with 5200ml of a mixture of ethanol and tert-butanol, mix at 45℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 65℃ for 15 hours.

Embodiment 10 Paclitaxel cholesterol compound

Take 5.0g of paclitaxel and 7.51g of cholesterol in a rotary evaporator, dissolve with 5200ml of a mixture of ethanol and tert-butanol,, mix at 45℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 65℃ for 15 hours.

Embodiment 11 Paclitaxel cholesterol compound

Take 5.0g of paclitaxel and 9.10g of cholesterol in a rotary evaporator, dissolve with 5200ml of a mixture of ethanol and tert-butanol,, mix at 45℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 65℃ for 15 hours.

Embodiment 12 Paclitaxel/ 7-dehydrocholesterol compound

Take 5g of paclitaxel, add 0.55g of 7-dehydrocholesterol in a rotary evaporator, dissolve with 500ml of acetone, mix at 45℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 65℃ for 15 hours.

Embodiment 13 Paclitaxel/ 7-dehydrocholesterol compound

Take 5g of paclitaxel, add 4.85g of 7-dehydrocholesterol in a rotary evaporator, dissolve with 500ml of acetone, mix at 45℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 65℃ for 15 hours.

Embodiment 14 Paclitaxel/ 7-dehydrocholesterol compound

Take 5g of paclitaxel, add 9.70g of 7-dehydrocholesterol in a rotary evaporator, dissolve with 500ml of acetone, mix at 45℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 65℃ for 15 hours.

Embodiment 15 Paclitaxel/ ergosterol compound

Take 5g of paclitaxel and 0.54g of ergosterol in a rotary evaporator together, dissolve with 500ml of acetone, mix at 45℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 65℃ for 15 hours.

Embodiment 16 Paclitaxel/ ergosterol compound

Take 5g of paclitaxel and 4.83g of ergosterol in a rotary evaporator, dissolve with 500ml of acetone, mix at 45℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 65℃ for 15 hours.

Embodiment 17 Paclitaxel/ ergosterol compound

Take 5g of paclitaxel and 9.10g of ergosterol in a rotary evaporator, dissolve with 500ml of acetone, mix at 45℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 65℃ for 15 hours.

Embodiment 18 Paclitaxel/ cholic acid compound

Take 4g of paclitaxel each in 5 portions, add 0.5g, 2.0g, 3.0g, 4.6g and 7.70g of cholic acid separately in a rotary evaporator, dissolve with 1000ml of acetone each, mix at 45℃ for 1 hour, then remove the solvent by rotary evaporation method, vacuum drying at 65℃ for 15 hours.

Embodiment 19 Submicron emulsion prepared with paclitaxel/ steroid compound as the intermediate carrier

Dissolve 25g of glycerol with approximate 720ml of water for injection, heat to 40-60℃, add 15g of refined soybean lecithin and 30g of poloxamer (188) to a blender, obtain the homogeneous water phase, keep at its temperature; take a quantity of paclitaxel/ steroid compound from embodiment 1 to 18 (equivalent to 500mg of paclitaxel), add 200ml of soybean oil, heat to 40-60℃, obtain the oil

phase. Mix the water phase slowly into the oil phase, stir to producing uniform colostrum. Transfer the colostrum to a homogenizer several times, adjusting pH to 4.0-6.0 with 0.1mol/L of hydrochloric acid, collect all emulsion, sterilize at 115℃ for 30 mins, obtain submicron emulsion with 0.5mg/ml of drug loading. The average particle size is 125nm~150nm detected by Lazer particle size analyzer .

Embodiment 20 Submicron emulsion prepared with paclitaxel/ steroid compound as the intermediate carrier

Dissolve 12.5g of glycerol with approximate 300ml of water for injection, heat to 50℃, add 7.5g of refined egg yolk lecithin and 15g of poloxamer (188) to a blender, obtain homogenous water phase, keep at its temperature; take a quantity of paclitaxel/ steroid compound from embodiment 5, 13 and 16 (equivalent to 500mg of paclitaxel), add 125ml of a mixture containing soybean oil and MCT by volume, heat to 50℃, obtain the oil phase. Mix the water phase slowly into the oil phase, stir at high speed to producing uniform colostrum. Transfer the colostrum to a homogenizer several times, adjusting pH to 4.0-6.0 with 0.1mol/L of hydrochloric acid, add water to 500ml, collect all emulsion, sterilize at 115℃ for 30 mins, obtain submicron emulsion with 1.0mg/ml of drug loading. The average particle size is 230nm~250nm detected by Lazer particle size analyzer.

Embodiment 21 Submicron emulsion prepared with paclitaxel/ steroid compound as the intermediate carrier

Dissolve 12.5g of glycerol with approximate 300ml of water for injection, heat to 55℃, add 7.5g of refined soybean lecithin and 15g of poloxamer (188) to a blender, obtain homogenous water phase, keep at its temperature; take a quantity of paclitaxel/ steroid compound from embodiment 1 to 18 (equivalent to 600mg of paclitaxel), add 125ml of soybean oil, heat to 55℃, obtain the oil phase. Mix the water phase slowly into the oil phase, stir at high speed to producing uniform colostrum. Transfer the colostrum to a homogenizer several times, adjusting pH to 4.0-6.0 with 0.1mol/L hydrochloric acid, add water to 500ml, collect all emulsion, sterilize at 115℃ for 30 mins, obtain submicron emulsion with 1.2mg/ml of drug loading. The average particle size is 125nm~133nm detected by Lazer particle size analyzer.

Embodiment 22 Submicron emulsion prepared with paclitaxel/ steroid compound as the intermediate carrier

Dissolve 12.5g of glycerol with approximate 300ml of water for injection, heat to 60℃, add 7.5g of refined soybean lecithin and 15g of poloxamer (188) to a blender, stir at high speed to producing homogenous water phase, keep at its temperature; take a quantity of paclitaxel/ steroid compound from embodiment 1 to 18 (equivalent to 1000mg of paclitaxel) respectively, add 150ml of soybean oil, heat to 60℃, obtain the oil phase. Mix the water phase slowly into the oil phase, stir at high speed to producing uniform colostrum. Transfer the colostrum quickly to a homogenizer several times, adjusting pH to 4.0-6.0 with 0.1mol/L of

hydrochloric acid, add water to 500ml, collect all emulsion, sterilize at 115℃ for 30 mins, obtain submicron emulsion with 2.0mg/ml of drug loading. The average particle size is 128nm~141nm detected by Lazer particle size analyzer.

Embodiment 23 Dry emulsion prepared with paclitaxel/ steroid compound as the intermediate carrier

Take a quantity of non-sterilized submicron emulsion from embodiment 20 to 22, dissolve with 3% (W/V) of mannitol, sterilze and filter through 0.22μm of microporous membrane, freeze to drying.

Embodiment 24 Self-microemulsion prepared with paclitaxel/ steroid compound as the intermediate carrier

Take a quantity of paclitaxel/ steroid compound (equivalent to 100mg of paclitaxel) form embodiment 1-18 respectively, add 5ml of MCT, mix to dissolve, add 1.6ml of PEG400 and 0.5ml of Tween-80, mix well, obtain the uniform and transparent self-microemulsifying system.

Take 10ml of self-microemulsifying system as mentioned above, add 5% of glucose injection to 50ml, and then obtain mciroemulsion promptly with particle size less than 100nm.

Embodiment 25 Capsules prepared with paclitaxel/ steroid compound as the intermediate carrier

Take a quantity of paclitaxel/ steroid compound (equivalent to 250mg of paclitaxel) form embodiment 1-18 respectively into No.2 hard capsules.

Embodiment 26 Tablets prepared with paclitaxel/ steroid compound as the intermediate carrier

Take a quantity of paclitaxel/ steroid compound (equivalent to 500mg of paclitaxel) form embodiment 1-18 respectively, add 500mg of microcrystalline cellulose, 500mg of lactose, and an amount of magnesium stearate, mix well, adjust the punch to compressing 100-250mg of tablets in specification.

Test Cases

Test Case 1:

Technical solution validation as disclosed in China Patent Claim 200810168213.X

Carry out the preferred lipid materials and its amount as claimed in *China Patent Claim 200810168213.X*, use soya bean lecithin and cholesterol as lipid materials and weight ratio of drug and lipid materials stands 1:1.85~18.5, prepare the reference compound, investigate the solubility in soybean oil (calculated as paclitaxel), compared with uncombined free paclitaxel.

Table 1 Validation results from the compound as disclosed in Patent

Compound	Amount ratio of paclitaxel/ lipid materials		Solubility in soybean oil (mg/ml)
	Molar ratio	Weight ratio	
'aclitaxel/ 'hospholipids	1:2	1:1.85	0.68
	1:4	1:3.70	0.75
	1:6	1:5.55	2.12
	1:10	1:9.23	2.32
	1:20	1:18.46	2.25
Paclitaxel/ Cholesterol	1:4	1:1.82	9.04
	1:6	1:2.73	8.87
	1:10	1:4.53	9.15
	1:20	1:9.06	8.75
	1:40	1:18.12	8.69
Paclitaxel Reference	/	/	0.27

The results has been shown that the amount of phospholipids is 5.55 times that of paclitaxel, solubility intends to be stable and controlled at 2.12 ~ 2.32mg/ml; while the amount of cholesterol is 1.82~18.12 times the weight of paclitaxel, the solubility is controlled at 8.75 ~ 9.15mg/ml, it is suggested that using cholesterol as

lipid materials is superior to phospholipids in the aspect of improving the solubility of drug substance in oil and the amount of cholesterol is expected to be further decreased and optimized.

Test Case 2

Influencing factors analysis of paclitaxel/ steroid compound in oil

Amount ratio of paclitaxel and steroid

Take 4g of paclitaxel, add cholesterol, 7-dehydrocholesterol and ergosterol respectively in accordance with the amount ratio as stated in the table 2 below, move to a rotary evaporator together, dissovle with 500ml of acetone each, mix at 45℃ for 1 hour then remove the solvent by the rotary evaporation method, vacuum drying at 65℃ for 15 hours. Take a quantity of compound, add 20g of soybean oil for injection used, heat in water bath for 1 hour and shake, obtain the saturated solution. Filter the solution, take a quantity of the successive filtrate, add absolute ethanol to volume, inject 20μl into the liquid chromatography, using Kromasil-C18 (250mm×4.6mm,5μm) as the column, acetonitrile-water (54:46) as the mobile phase, flow rate at 1.0ml/min and detection wavelength is 230nm, calculate the solubility of paclitaxel in vegetable oil, see results in the table below.

Table 2 Effect of amount ratio of paclitaxel and steroid on the solubility of the compound in oil

Steroid lipid materialss	Amount ratio of paclitaxel and steroid		Solubility in soybean oil (mg/ml)
	Molar ratio	Weight ratio	
Cholesterol	5:1 (1:0.2)	1:0.091	7.66
	4:1 (1:0.25)	1:0.112	8.25
	3:1 (1:0.33)	1:0.151	8.56
	2:1 (1:0.5)	1:0.225	9.14
	1:1	1:0.453	9.49
	1:2	1:0.906	9.06
	1:4	1:1.813	9.04
7-dehydrocholesterol	5:1 (1:0.2)	1:0.090	7.47
	4:1 (1:0.25)	1:0.113	7.93
	3:1 (1:0.33)	1:0.150	8.77
	2:1 (1:0.5)	1:0.225	9.21
	1:1	1:0.450	8.85
	1:2	1:0.901	8.69
	1:4	1:1.802	9.12
Ergosterol	5:1 (1:0.2)	1:0.093	7.23
	4:1 (1:0.25)	1:0.116	8.50
	3:1 (1:0.33)	1:0.155	9.26
	2:1 (1:0.5)	1:0.232	8.78
	1:1	1:0.465	8.59

	1:2	1:0.929	9.11
	1:4	1:1.858	8.73
Paclitaxel reference	/	/	0.27

The results have been shown that molar ratio of paclitaxel and steroid stands 1: 0.2~4, that is the maximum amount of steroid less than 4 times the molar of paclitaxel, the maximum solubility of drug in oil is obtained.

Paclitaxel concentration

Using acetone as the solvent, amount molar ratio of paclitaxel and cholesterol is 1:1, the reaction temperature at 35°C, the reaction time is 0.5 h, it is to investigated the effect of paclitaxel concentration of 16.0, 8.0, 4.0, 2.0, 1.0 and 0.5mg/ml on the compound preparation. The results have been shown the concentration of paclitaxel has no obvious effect on the solubility of compound in oil, see the table below.

Table 3 Effect of paclitaxel concentration on solubility of cholesterol compound in oil

Paclitaxel concentration (mg/mL)	Solubility in soybean oil (mg/ml)
16. 0	8. 89
8. 0	9. 21
4. 0	9. 58
2. 0	9. 47

1.0	9.39
0.5	8.44

Test Case 3 Paclitaxel cholesterol compound in DSC curves

Test samples:

- Paclitaxel
- Cholesterol
- Paclitaxel cholesterol compound (compound as prepared in table 2 of Test Case 2 with the molar ratio of 4:1, 3:1, 2:1, 1:1, 1:2, 1:4);
- Physical mixture of paclitaxel and cholesterol (take a quatlity of paclitaxel and cholesterol with the same molar ratio, mix well, intended for comparative research)

Method: carry out the differential scanning calorimetry (DSC) to detremine the characteristics of DSC curves, temperature at 25~300℃, heating rate is 10℃/min, nitrogen flow rate is 60ml/min; DSC curves have been shown in Figure 1.

Result: endothermic melting peak of paclitaxel is at 225.7℃ and that of cholesterol is at 150.9℃. In the preparation of physical mixture in different proportions, the endothermic characteristics of cholesterol has not been changed with the melting point at 149-150℃; whereas the endothermic peak of paclitaxel has a little shift but melting feature remains, it is just a simple physical mixed. The shift reason of melting peak obtained with paclitaxel in the physical mixture as

following, the melting point of cholesterol is lower than that of paclitaxel, after cholesterol melting, it has affected the dispersed state of drug, which will change the melting characteristicsof paclitaxel.

In the preparation of compound in different proportions, the melting characteristics of cholesterol has shift obviously, and the endothermic peak of paclitaxel has disappeared completely, which indicates that both have fully compounded.

Test Case 4 Solubility research on paclitaxel cholesterol compound in different oil phases

Take a quantity of paclitaxel cholesterol compound as described in table 2 of Test Case 2, add a mixture of soybean oil for injection, MCT and soybean oil/MCT (1:1), heat to 60℃, mix well to dissolve, heat in the water bath at 60℃ for 1 hour, shaking constantly during the process, obtain the saturated solution. Filter the solution, take a quantity of the successive filtrate, dilute with absolute ethanol, carry out the HPLC method, calculate the solubility of compound in oil; take a quantity of paclitaxel, repeat the operation.. Compared the solubility of compound and paclitaxel, the results have been proved that the solubility in oil can be improved when the drug combining with cholesterol in different proportions, see the table below:

Table 4 Results of drug solubility in vegetable oil (mg/ml)

Drug/Cholesterol	5: 1	4: 1	3: 1	2: 1	1: 1	1: 2	1: 4	Paclitaxel

Soybean oil	7.66	8.25	8.56	9.14	9.49	9.06	9.04	0.27
MCT	25.25	26.21	26.47	26.45	23.54	27.69	27.69	2.37
Mixed oil*	15.51	13.87	14.02	16.56	15.50	16.89	16.89	1.36

Note: mixed oil* is a mixture of soybean oil and MCT (volume ratio of 1:1)

Test Case 5 Solubility of paclitaxel cholesterol compound in n-octanol

Take a quantity of paclitaxel cholesterol described in table 2 into 50ml of conical flask, add 10g of n-octanol, shake at 25℃ in the thermostatic oscillator for 24 hours, obtain the saturated solution. Take 5ml of the solution, filter by 0.45μm of membrane, take an amount of the successive filtrate, dilute with absolute ethanol, carry out the ultraviolet spectrophotometry method, calculate the apparent solubility of physical mixture and cholesterol in n-octanol, compared the results with paclitaxel. The results have been proved that the solubility in n-octanol can be improved when the drug combining with cholesterol in different proportions, see the table below:

Table 5 Results of drug solubility in n-octanol (mg/ml, 25℃)

Drug/Cholesterol	4: 1	3: 1	2: 1	1: 1	1: 2	1: 4	Paclitaxel
Solubility	23. 59	24. 28	20. 70	25. 83	28. 32	32. 41	8. 86

Test Case 6 X-ray diffraction (XRD) analysis of paclitaxel cholesterol compound

Test samples: paclitaxel, cholesterol, paclitaxel cholesterol compound (sample from embodiment 5), physical mixture of paclitaxel and cholesterol (molar ratio of 1:1).

Detection condition: Cu-K target, 40kV of tube voltage, 200MA of tube flow, diffraction range is $3°<2\theta<60°$.

Results: diffraction results are shown in Figure 2.

The results have been clearly stated that characteristic diffraction peaks of paclitaxel in No. 2, 4, 7, diffraction angle (2θ) at 5.480, 8.840 and 12.180, peak intensify is 23567, 11319, 13277; the strongest diffraction peak of cholesterol in No.1, diffraction angle (2θ) at 5.160, peak intensify is 35506; both characteristic diffraction peaks have been obviously shown in the diffraction spectrum, that is the sum profile of paclitaxel and cholesterol profile; but the diffraction spectrum of compound has been changed. The characteristic diffraction peaks intensify of paclitaxel and cholesterol have greatly weaken or peaks have disappeared, new characteristic diffraction peaks have been shown at angle of 15.240, 16.759, 17.160 and 17.960, diffraction peak intensity is 4492, 3588, 2604 and 3186, lower than that of paclitaxel and cholesterol separately. It is indicated that paclitaxel is dispersed at microcrystalline or amorphous state in the compound.

Test Case 7 Infrared spectrometry (IR) analysis of paclitaxel cholesterol compound

Take paclitaxel, cholesterol, paclitaxel cholesterol compound respectively (sample with molar ratio of 1:1 from embodiment 5), physical mixture of paclitaxel and cholesterol (molar ratio of 1:1), using potassium bromide to compress, carry out infrared spectroscopy method in the range of 400~4000 cm-1, the results have been shown in Figure 3.

IR spectrum has shown that the main absorption peak of paclitaxel is carbonyl group C = 0 which has two splitting peaks at 1733.8 cm-1 and 1714.4 cm-1, acylamino group has the carbonyl peak at 1646.4 cm-1 as well as stretching vibration absorption peak of oxhydryl group O-H at 3300-3500 cm-1; in chloesterol IR sperum, the characteristic absorption peaks are the staurated C-H bonding strenching vibration peaks at 2933.1cm-1, 2901.0cm-1 and 2866.4cm-1 and strenching vibration absorption peak of oxhydryl group at 3402.3cm-1; the IR specrum of the physical mixture is

essentially the sum of absorption peaks obtained with cholesterol and paclitaxel; the charcteristic absorption peaks of the compound has been changed, that is the characteristic peaks of carbonyl group and acylamino group obtained in paclitaxel have been changed, two splitting peaks of carbonyl group convert into a blunt peak with strong absorption at 17247cm-1, the peak shape of carbonyl group in acylamino group has been blunted at 1646.4 cm-1; for cholesterol, absorption peak shape of oxhydryl group has been widened at 3432.9 cm-1 in cholesterol and absorption intensify has been enhanced. It is indicated that carbonyl group of paclitaxel has reacted with oxhydryl group of cholesterol which could be produced a new compound.

Test Case 8 UV absorption spectroscopy (UV) and HPLC chromatographic peaks study of cholesterol compound

Dissolve paclitaxel and paclitaxel cholesterol compound (sample with the molar ratio of 1:1 from embodiment 5) separately with absolute ethanol, using absolute ethanol as blank, carry out UV scanning method in the range of 200~400nm, the results have been shown in Figure 4-1. It is concluded that the UV characteristics of paclitaxel complies with that of paclitaxel cholesterol compound which indicates the chromophore group of paclitaxel in the compound has not been changed, that is two components convert into compound by the intermolecular forces reaction without forming new chemical bonds.

Inject 20μl of ethanol solution of paclitaxel and paclitaxel cholesterol compound described above into the liquid chromatography, using Kromasil-C18 (250mm × 4.6mm, 5μm) as the column, acetonitrile - water (54:46) as the mobile phase, flow rate at 1.0ml / min, detection wavelength is 230nm, record the retention time, results have been shown in Figure 4-2. It is concluded that the retention time of the main peak obtained with paclitaxel complies with that of paclitaxel cholesterol compound, which indicates the free paclitaxel can be ionized from the compound in protonated ethanol solution.

Test Case 9 Stability study of paclitaxel cholesterol compound

Take solid powder of drug steroid compound prepared from embodiment 1-18, store at 25℃, sampling periodically and examine the appearance changes. Add absolute ethanol into the solution with suitable concentration, inject 20μl into HPLC, using Kromasil C-18 (250mm× 4.6mm, 5μm) as the column, acetonitrile-water (54:46) as the mobile phase, flow rate at 1.0ml/min, detection wavelength is 230nm, determine the

content and impurities. The results indicate that there is no significant changes in appearace, content and impurity and it has stable quality compared to the intitial.

Table 6 Stability of paclitaxel steroid compound in long-term storage

Temperature/Time		Appearance	Content(%)	Degradated products (%)
25℃	0 month	White powder	98.05~102.10	ND
	1 month	White powder	98.25~102.23	<0.2
	3 months	White powder	97.78~101.74	<0.3
	6 months	White powder	98.90~102.36	<0.3
	9 months	White powder	98.55~101.76	<0.2
	12 months	White powder	98.36~101.87	<0.2
	24 months	White powder	98.71~101.88	<0.2

Test Case 10

Test Case 10-1 Paclitaxel steroid compound

Experimental compound 1-6: according to the technical requirements of the present invention, use cholesterol, 7-dehydrocholesterol and ergosterol to preparing the paclitaxel steroid compound with molar ratio of 1:1-1:4. The detailed procedure is as following, take paclitaxel and steroid into a triangle flask, dissolve with 2000ml of acetone, mix at 40℃ for 1 hour, remove the solvent by rotary evaporation method, vacuum drying at 40℃ for 24 hours.

Reference compound 1-4: according to the technical requirements of Patent 200810168212.5, use soybean lecithin and cholesterol as lipid materials to preparing 4 groups of reference compound by the same method used for comparative study, wherein the molar ratio of paclitaxel phospholipids compound is 1:6 and 1:10, while the molar ratio of paclitaxel cholesterol compound is 1:10 and 1:20. As detailed in the table below.

Table 7 Composition of paclitaxel cholesterol compound

Compound No./lipid materials	Amount of paclitaxel	Amount of lipid materials	Paclitaxel/ lipid materials	
			Molar ratio	Weight ratio
Experimental compound 1/ cholesterol	8.0 g	3.6 g	1:1	1:0.45
Experimental compound 2/ cholesterol	8.0 g	7.2 g	1:2	1:0.90
Experimental compound 3/ 7-dehydrocholesterol	3.0 g	1.35g	1:1	1:0.45
Experimental compound 4/ 7-dehydrocholesterol	3.0 g	5.406g	1:4	1:1.80

Experimental compound 5/ ergosterol	3.0g	1.40g	1:1	1:0.46
Experimental compound 6/ ergosterol	3.0g	5.58g	1:4	1:1.86
Reference compound 1/ phospholipids	3.0 g	16.65g	1:6	1:5.55
Reference compound 2/ phospholipids	3.0 g	6.78g	1:10	1:9.23
Reference compound 3/ cholesterol	3.0g	13.5g	1:10	1:4.50
Reference compound 4/ cholesterol	3.0g	27.18g	1:20	1:9.06

Test Case 10-2 Paclitaxel submicron emulsion prepared with paclitaxel cholesterol compound as the intermediate carrier

[Formualtion]

Ingredients	submicron emulsion1	submicron emulsion 2	submicron emulsion 3	submicron emulsion 4
Experimental compound 1*	145mg	290mg	580mg	1160mg
Egg yolk lecithin	2g	2. 4g	3g	3g
Poloxamer (188)	1g	2g	4g	6g
Glycerol	5g	5g	5g	5g
Soybean oil	40ml	40ml	50ml	50ml
Add water for injection to	200ml	200ml	200ml	200ml
Total	200ml	200ml	200ml	200ml

*Experimental compound 1 is the compound with paclitaxel/ cholesterol weight ratio of 1:0.45 prepared from embodiment 1.

[Preparation]

▶take 130-140ml of water for injection, add egg yolk lecithin, Poloxamer (188) and glycerol according to formualtion, transfer to a blender to disperse, obtain homogeneous aqueous phase, heat to 40℃ and keep the temperature;

▶ take a quantity of soybean oil according to formualtion, preheat to 40℃, take paclitaxel cholesterol compound 1 prepared from embodiment 1, dissolve with preheated soybean oil, transfer to a blender to disperse, obtain homogeneous oil phase;

▶ add the aqueous phase slowly into the oil phase with constantly stirring, mix at 10,000 rpm / min for 5mins, obtain a homogeneous colostrum, transfer quickly into high-pressure homogenizer, homogenizer 6 times, collect all of emulsion, adjusting pH with 0.1mol/L of hydrochloric acid to 4.0 ± 0.5, add water to 200ml, shake and pack, sterilize at 115℃ for 30 minutes,.

For submicron emulsion 1-4, the amount of emulsifier (egg yolk lecithin) is 1.0% (g/ml), 1.2% (g/ml), 1.5% (g/ml) and 1.5% (g/ml) of total amount of submicron emulsion, the amount of cosurfactant Poloxamer (188) is 0.5% (g/ml), 1.0% (g/ml), 2.0% (g/ml) and 3.0% (g/ml) of total amount of submicron emulsion, and the drug loading of paclitaxel is 0.5mg/ml, 1.0mg/ml, 2.0mg/ml, 4.0mg/ml. Determined by Laser particle size analyzer, the average particle size of 4 groups emulsion is 225nm, 233 nm, 245nm and 230nm.

Test Case 10-3 Paclitaxel submicron emulsion prepared with paclitaxel cholesterol compound as the intermediate carrier

[Formualtion]

Ingredients	submicron emulsion 5	submicron emulsion 6	submicron emulsion 7	submicron emulsion 8
Experimental compound 2*	190mg	380mg	760mg	1520mg
Egg yolk lecithin	2g	2. 4g	3g	3g
Poloxamer(188)	2. 4g	4g	4g	6g
Glycerol	5g	5g	5g	5g
Vitamin E	/	/	/	40mg
Soybean oil	40ml	40ml	50ml	50ml
Add water for injection to	200ml	200ml	200ml	200ml
Total	200ml	200ml	200ml	200ml

*Experimental compound 2 is the compound with paclitaxel cholesterol compound weight ratio of 1:0.90 prepared from embodiment 1.

[Preparation]

►take 130-140ml of water for injection, add Poloxamer (188) and glycerol according to formualtion, transfer to a blender to disperse, obtain homogeneous aqueous phase, heat to 80℃ and keep the temperature;

► take a quantity of soybean oil according to formualtion, preheat to 80℃, take compound 2, egg yolk lecithin and vitamin E according to formulation, dissolve with preheated soybean oil, transfer to a blender to disperse, obtain homogeneous oil phase;

► add the aqueous phase slowly into the oil phase with constantly stirring, 20,000 rpm / min for 5mins, obtain a homogeneous colostrum, transfer quickly into high-pressure homogenizer, homogenizer 6 times, collect all of emulsion, adjusting pH with 0.1mol/L of hydrochloric acid to 5.5 ± 0.5, add water to 200ml, shake and pack, sterilize at 115℃ for 30 minutes.

For submicron emulsion 5-8, the amount of emulsifier (egg yolk lecithin) is 1.0% (g/ml), 1.2% (g/ml), 1.5% (g/ml) and 1.5% (g/ml) of total amount of submicron emulsion, the amount of cosurfactant Poloxamer (188) is 1.2% (g/ml), 2.0% (g/ml), 2.0% (g/ml) and 3.0% (g/ml) of total amount of submicron emulsion, and the drug loading of paclitaxel is 0.5mg/ml, 1.0mg/ml, 2.0mg/ml, 4.0mg/ml. Determined by Laser particle size analyzer, the average particle size of 4 groups emulsion is 246nm, 262 nm, 231nm, 242nm.

Test Case 10-4 Paclitaxel submicron emulsion prepared with paclitaxel cholesterol compound as the intermediate carrier

[Formualtion]

Ingredients	submicron emulsion 9	submicron emulsion 10	submicron emulsion 11	submicron emulsion 12

Experimental compound 1*	145mg	290mg	580mg	1450mg
Poloxamer(188)	4g	4g	4g	4g
Glycerol	5g	5g	5g	5g
Vitamin E	/	/	/	40mg
MCT	40ml	40ml	50ml	50ml
Add water for injection to	200ml	200ml	200ml	200ml
Total	200ml	200ml	200ml	200ml

*Experimental compound 1 is the compound with paclitaxel cholesterol weight ratio of 1:0.45 prepared from embodiment 1.

[Preparation]

The same procedure as Test Case 10-3, wherein adjusting pH to 5.0 ± 0.5.

For submicron emulsion 9-12, the amount of emulsifier (soybean lecithin) is 1.2% (g/ml), 1.2% (g/ml), 1.2% (g/ml) and 1.5% (g/ml) of total amount of submicron emulsion, the amount of cosurfactant Poloxamer (188) is 2.0% (g/ml) of total amount of submicron emulsion, and the drug loading of paclitaxel is 0.5mg/ml, 1.0mg/ml, 2.0mg/ml and 5.0mg/ml. Determined by Laser particle size analyzer, the average particle size of 4 groups emulsion is 165nm, 153nm, 127nm, 138nm.

Test Case 10-5 Paclitaxel submicron emulsion prepared with paclitaxel

cholesterol compound as the intermediate carrier

[Formualtion]

Ingredients	submicron emulsion 13	submicron emulsion 14	submicron emulsion 15	submicron emulsion 16
Experimental compound 1*	290mg	435mg	580mg	1450mg
soybean lecithin	2. 4g	2. 4g	2. 4g	4. 0g
Poloxamer(188)	3g	3g	4g	4g
Glycerol	5g	5g	5g	5g
Oil mixture**	40ml	40ml	40ml	50ml
Add water for injection to	200ml	200ml	200ml	200ml
Total	200ml	200ml	200ml	200ml

*Experimental compound 1 is the compound with paclitaxel cholesterol weight ratio of 1:0.45 prepared from embodiment 1.

**Oil mixture is a mixture of soybean oil and MCT (volume ratio of 1:1).

[Preparation]

The same procedure as embodiment 3, wherein adjusting pH to 4.5 ± 0.5.

For submicron emulsion 13-16, the amount of emulsifier (soybean lecithin) is 1.2% (g/ml), 1.2% (g/ml), 1.2% (g/ml) and 2.0% (g/ml) of total amount of submicron emulsion, the amount of cosurfactant Poloxamer (188) is 1.5%(g/ml), 1.5%(g/ml), 2.0%(g/ml) and 2.0% (g/ml) of total amount of submicron emulsion, and the drug loading of paclitaxel is 1.0mg/ml, 1.5mg/ml, 2.0mg/ml and 5.0mg/ml. Determined by Laser particle size analyzer, the average particle size of 4 groups emulsion is 145nm, 138nm, 133nm, 146nm.

Test Case 10-6 Paclitaxel submicron emulsion prepared with paclitaxel

cholesterol compound as the intermediate carrier

[Formualtion]

Ingredients	submicron emulsion 17	submicron emulsion 18	submicron emulsion 19	submicron emulsion 20
Experimental compound 2*	190mg	380mg	760mg	1520mg
egg yolk lecithin	3. 0g	3. 0g	4. 0g	6. 0g
Poloxamer(188)	4g	4g	6g	6g
Glycerol	5g	5g	5g	5g
Oil mixture**	30ml	40ml	50ml	60ml
Add water for injection to	200ml	200ml	200ml	200ml
Total	200ml	200ml	200ml	200ml

*Experimental compound 2 is the compound with paclitaxel cholesterol weight ratio of 1:0.90 prepared from embodiment 1.

**Oil mixture is a mixture of soybean oil and MCT (volume ratio of 1:1).

[Preparation]

The same procedure as embodiment 2, wherein adjusting pH to 5.5 ± 0.5.

For submicron emulsion 17-20, the amount of emulsifier (egg yolk lecithin) is 1.5% (g/ml), 1.5% (g/ml), 2.0% (g/ml) and 3.0% (g/ml) of total amount of

submicron emulsion, the amount of cosurfactant Poloxamer (188) is 2.0%(g/ml), 2.0%(g/ml), 3.0%(g/ml) and 3.0% (g/ml) of total amount of submicron emulsion, and the drug loading of paclitaxel is 0.5mg/ml, 1.0mg/ml, 2.0mg/ml and 5.0mg/ml. Determined by Laser particle size analyzer, the average particle size of 4 groups emulsion is 255nm, 263nm, 285nm, 232nm.

Test Case 10-7 Paclitaxel submicron emulsion prepared with paclitaxel cholesterol compound as the intermediate carrier

[Formualtion]

Ingredients	submicron emulsion 21	submicron emulsion 22	submicron emulsion 23	submicron emulsion 24
Experimental compound 1*	145mg	290mg	580mg	1160mg
Fatty glyceride	3. 0g	4. 0g	/	/
Polyoxyethylene sorbitan fatty acid ester	/	/	4. 0g	6. 0g
Poloxamer(188)	3g	4g	4g	6g
Glycerol	5g	5g	5g	5g
Oleic acid	0.2g	0.2g	0.2g	0.2g
Oil mixture**	30ml	40ml	50ml	60ml
Add water for injection to	200ml	200ml	200ml	200ml
Total	200ml	200ml	200ml	200ml

*Experimental compound 1 is the compound with paclitaxel cholesterol weight ratio of 1:0.45 prepared from embodiment 1.

**Oil mixture is a mixture of soybean oil and MCT (volume ratio of 1:1).

[Preparation]

The same procedure as embodiment 3

For submicron emulsion 21 and 22, the amount of emulsifier (fatty glyceride) is 1.5% (g/ml) and 2.0% (g/ml) of total amount of submicron emulsion; for submicron emulsion 23 and 24, the amount of emulsifier (polyoxyethylene sorbitan fatty acid ester) is 2.0% (g/ml) and 3.0% (g/ml) of total amount of submicron emulsion; for submicron emulsion 21-24, the amount of cosurfactant Poloxamer (188) is 1.5%(g/ml), 2.0%(g/ml), 2.0%(g/ml) and 3.0% (g/ml) of total amount of submicron emulsion, and the drug loading of paclitaxel is 0.5mg/ml, 1.0mg/ml, 2.0mg/ml and 4.0mg/ml. Determined by Laser particle size analyzer, the average particle size of 4 groups emulsion is 145nm, 133nm, 126nm, 158nm.

Test Case 10-8 Submicron emulsion prepared with 7-dehydrocholesterol or ergosterol as the intermediate carrier

[Formualtion]

Ingredients	submicron emulsion 25	submicron emulsion 26	submicron emulsion 27	submicron emulsion 28
Experimental	290mg	/	/	/

compound 3*				
Experimental compound 4*	/	5604mg	/	/
Experimental compound 5*	/	/	292mg	/
Experimental compound 6*	/	/	/	5720mg
Soybean lecithin	2. 4g	2. 4g	2. 4g	4. 0g
Poloxamer(188)	3g	3g	4g	4g
Glycerol	5g	5g	5g	5g
Oil mixture**	40ml	40ml	40ml	50ml
Add water for injection to	200ml	200ml	200ml	200ml
Total	200ml	200ml	200ml	200ml

*Experimental compound 3-6 is a mixture of paclitaxel 7-dehydrocholesterol compound and paclitaxel ergosterol compound prepared from embodiment 1.

**Oil mixture is a mixture of soybean oil and MCT (volume ratio of 1:1).

[Preparation]

The same procedure as embodiment 5

For submicron emulsion 25 and 28, the drug loading of paclitaxel is 1.0mg/ml. Determined by Laser particle size analyzer, the average particle size of 4 groups of emulsion is 143nm, 138nm, 141nm, 132nm.

Test Case 10-9 Paclitaxel submicron emulsion prepared with the reference compound as the intermediate carrier

Take the reference compound 1-4 prepared from Test Case 1, carry out the method with respect to the preparation of submicron emulsion as described in embodiment above, obtain submicron emulsion 29-32 with drug loading of 5, 1.0,

1.0 and 2.0mg/ml, used for comparative study.

The results of the detailed formulation, preparation method and encapsulation efficiency have been shown as following:

[Formualtion]

Ingredients	submicron emulsion 29	submicron emulsion 30	submicron emulsion 31	submicron emulsion 32
Reference compound 1*	655mg	/	/	/
Reference compound 2*	/	1023mg	/	/
Reference compound 3*	/	/	1100mg	/
Reference compound 4*	/	/	/	4024mg
Egg yolk lecithin	3g	3g	3g	3g
Poloxamer(188)	3g	3g	3g	3g
Glycerol	5g	5g	5g	5g
Soybean oil	40ml	40ml	50ml	50ml
Add water for injection to	200ml	200ml	200ml	200ml
Total	200ml	200ml	200ml	200ml

*Reference compound 1and 2 are two groups of reference compound with paclitaxel/ phospholipids weight ratio of 1:5.55 and 1:9.23 prepared from Test Case 1.

*Reference compound 3and 4 are two groups of the reference compounds with cholesterol/ paclitaxel phospholipids weight ratio of 1:4.50 and 1:9.06 prepared from Test Case 1.

[Preparation]

►take 130-140ml of water for injection, add egg yolk lecithin, Poloxamer (188) and glycerol according to formualtion, transfer to a blender to disperse, obtain homogeneous aqueous phase, heat to 40-80℃ and keep the temperature;

► take the quantity of soybean oil according to formualtion, preheat to 40-80 ℃, take paclitaxel cholesterol compound 1 prepared from embodiment 1, dissolve with preheated soybean oil, transfer to a blender to disperse, obtain homogeneous oil phase;

► add the aqueous phase slowly into the oil phase with constantly stirring, mix at 10,000-20,000 rpm / min for 5-10mins, obtain homogeneous colostrum, transfer quickly into high-pressure homogenizer, homogenizer 6 times, collect all of emulsion, adjusting pH with 0.1mol/L of hydrochloric acid to 4.5 ± 0.5, add water to 200ml, shake and pack, sterilize at 115℃ for 30 minutes.

The stability research and comparison of submicron emulsion prepared in Test Cases above

Take 28 groups of submicron emulsion from Test Cases 10-2 to 10-8 and 4 groups of submicron emulsion from Test Case 10-9, store at 4℃ for 12 months

respectively, sampling at 0, 6, 12 months, carry out the method as described below to examine the changes in appearance, particle size, content and impurity.

Characteristics: Visual examination, record oil droplets or layered appeared in the surface complying with the colour of submicron emulsion described.

Particle size: take submicron emulsion, examine the particle size using MASTER SIZER 2000 laser particle size analyzer (malvern).

Content and related substances: Take accurately the quantity of paclitaxel submicron emulsion, add anhydrous ethanol to demulsification, obtain the suitable concentration of the test solution. Inject accurately 20μl of the test solution into chromatography, carry out HPLC method, using Kromasil-C18(300mm×4.6mm , 5μm) as the column, acetonitrile - water (54:46) as the mobile phase, flow rate at 1.0ml/min, detection wavelength is 230nm, the column temperature is the room temperature, record the chromatogram, calculate the content of drug substance in emulsion with respect to the peak by the external standard method, caculate the content of impurity by normalization method.

Results: as detailed in the table below

Table 8 Comparative results of submicron emulsion stability

Sample No./drug loading	**Initial（examine within one week after preparation）**				**Stored at 4℃ for 12 months**			
	Appearance	**Particle size**	**content**	**impurity**	**Appearance**	**Particle size**	**content**	**impurity**
Emulsion 1 /0.5 mg/ml	Uniform emulsion	225nm	100.2%	0.33%	Uniform emulsion	221nm	99.7%	0.67%
Emulsion 2	Uniform	233nm	97.6%	0.31%	Uniform	245nm	97.3%	0.62%

/1.0mg/ml	emulsion				emulsion			
Emulsion 3 /2.0mg/ml	Uniform emulsion	245nm	98.5%	0.31%	Uniform emulsion	236nm	98.2%	0.64%
Emulsion 4 /3.0mg/ml	Uniform emulsion	230nm	97.6%	0.37%	Uniform emulsion	237nm	97.0%	0.93%
Emulsion 5 /0.5mg/ml	Uniform emulsion	246nm	99.5%	0.35%	Uniform emulsion	228nm	98.8%	0.52%
Emulsion 6 /1.0mg/ml	Uniform emulsion	262nm	100.3%	0.30%	Uniform emulsion	255nm	99.5%	0.61%
Emulsion 7 /2.0mg/ml	Uniform emulsion	231nm	98.1%	0.36%	Uniform emulsion	240nm	98.6%	0.57%
Emulsion 8 /4.0mg/ml	Uniform emulsion	242nm	99.6%	0.42%	Uniform emulsion	251nm	97.3%	1.01%
Emulsion 9 /0.5mg/ml	Uniform emulsion	165nm	98.8%	0.35%	Uniform emulsion	126nm	99.2%	0.56%
Emulsion 10 /1.0mg/ml	Uniform emulsion	153nm	101.6%	0.30%	Uniform emulsion	133nm	100.7%	0.52%
Emulsion 11 /2.0mg/ml	Uniform emulsion	127nm	102.2%	0.32%	Uniform emulsion	131nm	100.9%	0.55%
Emulsion 12 /5.0mg/ml	Uniform emulsion	138nm	99.8%	0.45%	Uniform emulsion	155nm	99.4%	1.24%
Emulsion 13 /1.0mg/ml	Uniform emulsion	145nm	97.4%	0.31%	Uniform emulsion	138nm	98.0%	0.57%
Emulsion 14 /1.5mg/ml	Uniform emulsion	138nm	100.3%	0.35%	Uniform emulsion	136nm	98.7%	0.65%
Emulsion 15 /2.0mg/ml	Uniform emulsion	133nm	99.2%	0.31%	Uniform emulsion	137nm	98.5%	0.63%
Emulsion 16 /5.0mg/ml	Uniform emulsion	146nm	98.5%	0.43%	Uniform emulsion	173nm	97.2%	1.68%

Emulsion 17 /0.5mg/ml	Uniform emulsion	255nm	97.8%	0.32%	Uniform emulsion	244nm	97.6%	0.57%
Emulsion 18 /1.0mg/ml	Uniform emulsion	263nm	99.2%	0.36%	Uniform emulsion	259nm	99.5%	0.53%
Emulsion 19 /2.0mg/ml	Uniform emulsion	285nm	100.4%	0.33%	Uniform emulsion	272nm	98.8%	0.61%
Emulsion 20 /5.0mg/ml	Uniform emulsion	232nm	101.4%	0.48%	Uniform emulsion	258nm	97.8%	1.76%
Emulsion 21 /0.5mg/ml	Uniform emulsion	145nm	98.7%	0.36%	Uniform emulsion	136nm	99.1%	0.59%
Emulsion 22 /1.0mg/ml	Uniform emulsion	133nm	98.2%	0.32%	Uniform emulsion	127nm	97.6%	0.57%
Emulsion 23 /2.0mg/ml	Uniform emulsion	126nm	101.1%	0.38%	Uniform emulsion	134nm	99.5%	0.56%
Emulsion 24 /4.0mg/ml	Uniform emulsion	158nm	98.8%	0.41%	Uniform emulsion	163nm	97.9%	1.25%
Emulsion 25 /1.0mg/ml	Uniform emulsion	143nm	99.5%	0.33%	Uniform emulsion	128nm	98.9%	0.56%
Emulsion 26 /1.0mg/ml	Uniform emulsion	138nm	97.8%	0.35%	Uniform emulsion	132nm	97.4%	0.58%
Emulsion 27 /1.0mg/ml	Uniform emulsion	141nm	99.4%	0.30%	Uniform emulsion	145nm	99.1%	0.61%
Emulsion 28 /1.0mg/ml	Uniform emulsion	132nm	98.6%	0.36%	Uniform emulsion	126nm	98.2%	0.54%
Emulsion 29 /0.5mg/ml	Uniform emulsion	253nm	97.5%	0.92%	Layered with floading oil	323nm	90.8%	7.63%
Emulsion 30 /1.0mg/ml	Layered	522nm	93.2%	6.27%	Oil-water separation	Not detected	Not detected	Not detected
Emulsion 31	Uniform	247nm	98.6%	0.62%	Uniform	263nm	94.7%	3.58%

/1.0mg/ml	emulsion				emulsion			
Emulsion 32 /2.0mg/ml	Uniform emulsion	266nm	98.9%	0.78%	Slightly layered	311nm	92.8%	4.64%

Submicron emulsion 1-28 prepared with steroidal compound as the intermediate carrier in the present invention, store in the refrigerator (4℃) for 12 months, compared with the initial. 1) For emulsion with 5.0mg/ml of drug loading, it is to be noted that the average particle size intends to be greater. The emulsion has not layered and particle size, appearance and content has not obviously changed, impurity increases but not exceeds 2.0%; 2) emulsion with 4.0mg/ml of drug loading has not layered and no obvious changes in particle size, appearance and content, impurity increases but not exceeds 1.3%; 3) emulsion with 3mg/ml of drug loading has not layered and no obvious changes in particle size, appearance and content, impurity not exceeds 1.0%; 4) emulsion with 2mg/ml or less than 2mg/ml of drug loading has no obvious changes in particle size, appearance and content, impurity less than 0.7%.

Submicron emulsions prepared with reference paclitaxel/ phospholipids compound as carrier, 1) the uniform emulsion (submicron emulsion 29) has been formed if the drug loading of 0.5mg/ml, placed for 6 months, there is no obvious changes in appearance and particle size and impurity increases to 3.0%, whereas placed for 12 months, particle size has significant changed and impurity increases to 7% or above, the content decreases with layered and floating oil; 2) if the drug loading increased into 1.0mg/ml, the uniform emulsion has not formed (submicron

emulsion 30), the drug crystallization and oil droplets has been appeared at the beginning.

Submicron emulsions prepared with reference paclitaxel/ cholesterol compound as carrier, 1) the uniform emulsion (sample 31-32) has been formed when drug loading of 1.0mg/ml and 2.0mg/ml, placed for 6 months, there is no obvious changes in appearance, particle size and content has not obviously changed and impurity increases but not exceeds 1.5%; 2) placed for 12 months, particle size has significant changed, content decreases and impurity increases to 3.58% and 4.64%, wherein emulsion with drug loading of 2.0mg/ml has slightly layered.

Claims

1. A kind of paclitaxel steroid compound, characterized in that it is the compound composed of paclitaxel and steroid with molar ratio of 1:0.2~4.

2. A kind of paclitaxel steroid compound as claimed in Claim 1, characterized in that the molar ratio of paclitaxel and steroid is 1:0.25~2.

3. A kind of paclitaxel steroid compound as claimed in Claim 2, characterized in that the molar ratio of paclitaxel and steroid is 1:0.33~1.

4. A kind of paclitaxel steroid compound as claimed in Claim 1-3, characterized in that steroid claimed is the natural steroids or its derivatives.

5. A kind of paclitaxel steroid compound as claimed in Claim 4, characterized in that the natural steroid claimed can be chosen from one kind of cholesterol, 7- dehydrocholesterol, lanosterol, sitosterol, brassicasterol, mycosterol, oysters steroid, stigmasterol, sitosterolum or ergosterol at least; and its derivative can be chosen from one kind of cholic acid, deoxycholic acid or anthropodesoxycholic acid at least.

6. A kind of paclitaxel steroid compound as claimed in Claim 1-5, characterized in that the antioxidant stabilizer has been used in the compound claimed.

7. A kind of paclitaxel steroid compound as claimed in Claim 6, characterized in that the antioxidant stabilizer should be chosen from one kind of sodium bisulfite, sodium metabisulfite, vitamin C, EDTA and its salts, vitamin E and its derivatives at least.

8. The preparation of paclitaxel steroid compound as claimed in Claim 1-7, characterized in that the preparation process involves the following steps:

a. mix paclitaxel and steroid lipid materials in proportion, add organic solvent to dissolve, add antioxidant stabilizer randomly;

b. stir at the suitable temperature, remove the organic solvent, vacuum drying.

9. The preparation as claimed in Claim 8, characterized in that during the step a), take quantity of paclitaxel and steroid, dissolve with different organic solvents and mix.

10. The preparation as claimed in Claim 8-9, characterized in that the organic solvent claimed can be chosen from one or several of dichloromethane, ethanol, methanol, benzyl alcohol, acetone, ethyl acetate, tetrahydrofuran or tert-butanol.

11. The preparation as claimed in Claim 8-9, characterized in that the organic solvent claimed can be chosen from one or several of ethanol, acetone, ethyl acetate or tetrahydrofuran.

12. A kind of paclitaxel steroid compound as claimed in Claim 1-7, characterized in that it is also provided with the preparation of oil-in-water submicron emulsion, dry emulsion, self-microemulsifying system or oral preparations.