An Anti-aging Composition and Its Preparation Method and Skin Care Application

Field of the Invention

This invention relates to the field of daily chemicals, and more particularly, to an anti-aging composition and its preparation method and skin care application.

Background of the Invention

Skin aging is mainly caused by two reasons, one is endogenous aging, and the other is exogenous aging. Endogenous aging mainly refers to the aging characteristics of the skin with the increase of age: more wrinkles, loose and yellowish skin, reduced luster, glow and smoothness, coarser texture, and more color spots; exogenous aging mainly refers to the acceleration of skin cell damage and natural aging due to staying up late, work stress, life anxiety, UV damage, and other reasons. In addition, there is another kind of expression wrinkles caused by personal expression habits, which cannot be avoided by self-consciousness. Because the skin is repeatedly stretched and relaxed by muscles, collagen is rearranged, eventually forming wrinkles over time. With the rapid development of the economy and the improvement of people's living standards, consumers are increasingly pursuing anti-aging cosmetics, especially effective ingredients, nursing efficacy, and safety. At present, there are many kinds of "anti-aging" raw materials on the market, but there are many problems such as insufficient efficacy, single function, poor absorption, and unstable efficacy components. For example, "retinol", a commonly used raw material in anti-

aging skin care products, is called "retinoids" family together with its many derivatives, including retinene, retinoic acid (i.e. "tretinoin"), retinyl esters (such as Retinyl palmitate, Retinyl acetate, Retinyl propionate, and Retinyl retinoate), β-Carotene, etc., and they are often applied in anti-aging products, such as face creams, essences, and eye creams. However, there are some problems in the application of retinol: ① Light instability: Because the Tetraene side chain of retinol is easy to accept the energy of light waves, its structure is easy to change and its activity is lost under UV irradiation; ② Activity decay: Experimental research shows that at room temperature, retinol, retinene, and retinyl ester standard solutions have different degrees of activity decay, of which retinol has the most serious decay; ③ Irritation to the skin: People with different skin types show different tolerance when using it and may experience discomfort such as erythema, peeling, and pain. It is found in the research in the literature (Fu P P, Cheng S H, Coop L, et al. Photoreaction, phototoxicity, and photocarcinogenicity of retinoids [J]. Journal of Environmental Science and Health Part C: Environmental Carcinogenesis & Ecotoxicology Reviews, 2003, 21(2): 165-197.) that, compared with retinol, Retinyl palmitate is more unstable under UV light, and UVB has a stronger photodegradation effect on retinoids, which has photosensitivity and phototoxicity; at the same time, its amount in skin care products is limited because of the safety risks of retinoids.

Conventional liposomes are liposomes (hollow) made of lecithin, ceramide, etc. They have a bilayer structure and the main raw materials are phospholipids and cholesterol. The hydrophilic head of phospholipid molecules extends into the aqua and the hydrophobic tail extends into the air. Cholesterol is inlaid between

phospholipid molecules, which has the function of regulating membrane fluidity and has an excellent moisturizing effect on the skin. Especially when it is coated with moisturizing substances such as hyaluronic acid and polyglucoside, it is a more excellent moisturizing substance. However, conventional liposomes have some problems, such as poor stability, low encapsulation efficiency of fat-soluble active substances, and low transdermal penetration rate.

On this basis, it is of great practical significance to develop a raw material for anti-aging skin care products with good stability, good transdermal absorption, and repair effects on skin tissue damage.

Summary of the Invention

To solve said technical problems, the invention provides an anti-aging composition and its preparation method and skin care applications. This anti-aging composition has good stability and transdermal absorption and can repair the skin tissue morphology, accelerate the renewal of elastic fibers, promote the generation of collagenous fibers, and achieve the efficacy of anti-aging and skin tightening.

Therefore, the technical scheme provided by the application is as follows:

In the first aspect, the invention provides an anti-aging composition.

According to the invention, said anti-aging composition is composed of anti-wrinkle composition, Soy lecithin, Glycerin, Caprylic/Capric triglyceride, Propylene glycol, Polysorbate 80, Palmitoyl pentapeptide-4, Dendrobium Officinale stem extract, and Aqua.

In the invention, Dendrobium Officinale stem extract is an aqueous extract. In some embodiments, the preparation method of said Dendrobium Officinale stem extract includes: mixing and extraction of Dendrobium Officinale stem and aqua, filtering, membrane separation, drying, crushing, and sterilization.

In the invention, the CAS No. of Caprylic/Capric triglyceride is 73398-61-5 and that of Arginine/Lysine polypeptide is 936616-33-0.

According to the invention, by weight percentage, said anti-aging composition is composed of 30.00wt%-70.00wt% anti-wrinkle composition, 0.50wt%-2.50wt% Soy lecithin, 2.50wt%-12.50wt% Glycerin, 0.25wt%-1.25wt% Caprylic/Capric triglyceride, 15.00wt%-35.00wt% Propylene glycol, 0.25wt%-1.25wt% Polysorbate 80, 0.01wt%-0.03wt% Palmitoyl pentapeptide-4, 0.10wt%-0.20wt% Dendrobium Officinale stem extract, and Aqua To100.

According to the invention, by weight percentage, the amount of anti-wrinkle composition in said anti-aging composition is 30.00wt%-70.00wt%, i.e., it can be 30.00wt%, 40.00wt%, 50.00wt%, 60.00wt%, or 70.00wt%, or any point value between the above values.

According to the invention, by weight percentage, the amount of Soy lecithin in said anti-aging composition is 0.50wt%-2.50wt%, i.e., it can be 0.50wt%, 1.00wt%, 1.50wt%, 2.00wt%, or 2.50wt%, or any point value between the above values.

According to the invention, by weight percentage, the amount of Glycerin is 2.50wt%-12.50wt%, i.e., it can be 2.50wt%, 5.00wt%, 7.50wt%, 10.00wt%, and 12.50wt%, or any point value between the above values.

According to the invention, by weight percentage, the amount of

Caprylic/Capric triglyceride in said anti-aging composition is 0.25wt%-1.25wt% i.e., it can be 0.25wt%, 0.50wt%, 0.75wt%, 1.00wt%, or 1.25wt%, or any point value between the above values.

According to the invention, by weight percentage, the amount of Propylene glycol in said anti-aging composition is 15.00wt%-35.00wt%, i.e., it can be 15.00wt%, 20.00wt%, 25.00wt%, 30.00wt%, or 35.00wt%, or any point value between the above values.

According to the invention, by weight percentage, the amount of Polysorbate 80 in said anti-aging composition is 0.25wt%-1.25wt% i.e., it can be 0.25wt%, 0.50wt%, 0.75wt%, 1.00wt%, or 1.25wt%, or any point value between the above values.

According to the invention, by weight percentage, said anti-aging composition is composed of 40.00wt%-60.00wt% anti-wrinkle composition, 0.75wt%-1.25wt% Soy lecithin, 2.50wt%-12.50wt% Glycerin, 0.50wt%-1.00wt% Caprylic/Capric triglyceride, 20.00wt%-30.00wt% Propylene glycol, and 0.50wt%-1.00wt% Polysorbate 80.

According to the invention, by the weight of the anti-wrinkle composition, said anti-wrinkle composition is composed of 0.001wt%-0.005wt% Arginine/Lysine polypeptide and Aqua To100.

According to the invention, by the weight of the anti-wrinkle composition, the amount of Arginine/Lysine polypeptide in said anti-wrinkle composition is 0.001wt%-0.005wt%, i.e., it can be 0.001wt%, 0.002wt%, 0.003wt%, 0.004wt%, or 0.005wt%, or any point value between the above values.

According to the invention, by weight percentage, the amount of Palmitoyl

pentapeptide-4 in said anti-aging composition is 0.01wt%-0.03wt%, i.e., it can be 0.01wt%, 0.02wt%, or 0.03wt%, or any point value between the above values.

According to the invention, said anti-aging composition is prepared by a preparation method comprising the following steps:

1) Preparation of oil phase and aqueous phase, said oil phase includes Soy lecithin, Glycerin, Caprylic/Capric triglyceride, Propylene glycol, and Palmitoyl pentapeptide-4; said aqueous phase includes Polysorbate 80, anti-wrinkle composition, and Dendrobium Officinale stem extract;

2) Add the oil phase to the aqueous phase for hydration to obtain a mixture of aqueous and oil phases;

3) Carry out high-speed shear on the mixture of aqueous and oil phases to obtain crude ethosomes;

4) Carry out high-pressure homogenization on crude alcohols to obtain ethosomes.

According to some embodiments of the invention, the oil phase preparation method in said Step 1) includes: weigh Soy lecithin, Glycerin, Caprylic/Capric triglyceride, and Propylene glycol, dissolve them, add Palmitoyl pentapeptide-4, and dissolve it also to obtain the oil phase.

According to some embodiments of the invention, the aqueous phase preparation method in said Step 1) includes: weigh Polysorbate 80, anti-wrinkle composition, and Dendrobium Officinale stem extract, and dissolve them in aqua to obtain the aqueous phase.

According to some embodiments of the invention, the hydration time in said

Step 2) is 10-30min, i.e., it can be 10min, 11min, 13min, 15min, 20min, 25min, or 30min, or any point value between the above values.

According to some embodiments of the invention, the high-speed shear speed in said Step 3) is 6,000-10,000rpm, i.e., it can be 6,000rpm, 7,000rpm, 8,000rpm, 9,000rpm, or 10,000rpm, or any point value between the above values.

According to some embodiments of the invention, the time of high-speed shear in said Step 3) is 1-5min, i.e., it can be 1min, 2min, 3min, 4min, or 5min.

According to some embodiments of the invention, the high-pressure homogenization pressure in said Step 4) is 600-1,000bar, i.e., it can be 600bar, 700bar, 800bar, 900bar, or 1,000bar.

According to some embodiments of the invention, the number of high-pressure homogenization in said Step 4) is 3-7 times, i.e., it can be 3, 4, 5, 6, or 7 times.

According to some embodiments of the invention, the oil phase preparation method in said Step 1) includes: weigh Soy lecithin, Glycerin, Caprylic/Capric triglyceride, and Propylene glycol, dissolve them, for example, at 50~60°C, dissolve them with magnetic stirring at 500~600rpm/min, stop heating after Soy lecithin is fully dissolved, add Palmitoyl pentapeptide-4, and dissolve it also to obtain the oil phase.

According to some embodiments of the invention, the aqueous phase preparation method in said Step 1) includes: weigh 0.25wt%-1.25wt% Polysorbate 80, 30.00wt%-70.00wt% anti-wrinkle composition, and 0.10wt%-0.20wt% Dendrobium Officinale stem extract, dissolve them in aqua, and in particular, dissolve them by ultrasonic into a homogeneous system to obtain the aqueous phase.

In the second aspect, the invention provides the preparation method for the anti-aging composition in the first aspect of the invention.

According to the invention, said preparation method includes the following steps:

1) Weigh 0.50wt%-2.50wt% Soy lecithin, 2.50wt%-12.50wt% Glycerin, 0.25wt%-1.25wt% Caprylic/Capric triglyceride, and 15.00wt%-35.00wt% Propylene glycol, dissolve them, add 0.01wt%-0.03wt% Palmitoyl pentapeptide-4, and dissolve it also to obtain the oil phase; weigh 0.25wt%-1.25wt% Polysorbate 80, 30.00wt%-70.00wt% anti-wrinkle composition, and 0.10wt%-0.20wt% Dendrobium Officinale stem extract and dissolve them in aqua to obtain the aqueous phase;

2) Add the oil phase to the aqueous phase for hydration for 10-30min to obtain a mixture of aqueous and oil phases;

3) Carry out high-speed shear at the speed of 6,000-10,000rpm for 1-5min on the mixture of aqueous and oil phases to obtain crude ethosomes;

4) Carry out 3-7 times of high-pressure homogenization under the pressure of 600-1,000bar on crude alcohols to obtain ethosomes.

According to some embodiments of the invention, the hydration time in said Step 2) is 10-30min, i.e., it can be 10min, 11min, 13min, 15min, 20min, 25min, or 30min, or any point value between the above values.

According to some embodiments of the invention, the high-speed shear speed in said Step 3) is 6,000-10,000rpm, i.e., it can be 6,000rpm, 7,000rpm, 8,000rpm,

9,000rpm, or 10,000rpm, or any point value between the above values.

According to some embodiments of the invention, the time of high-speed shear in said Step 3) is 1-5min, i.e., it can be 1min, 2min, 3min, 4min, or 5min.

According to some embodiments of the invention, the high-pressure homogenization pressure in said Step 4) is 600-1,000bar, i.e., it can be 600bar, 700bar, 800bar, 900bar, or 1,000bar.

According to some embodiments of the invention, the number of high-pressure homogenization in said Step 4) is 3-7 times, i.e., it can be 3, 4, 5, 6, or 7 times.

According to some embodiments of the invention, the oil phase preparation method in said Step 1) includes: weigh 0.50wt%-2.50wt% Soy lecithin, 2.5wt%-12.5wt% Glycerin, 0.25wt%-1.25wt% Caprylic/Capric triglyceride, and 15.00wt%-35.00wt% Propylene glycol, dissolve them, for example, at 50~60°C, dissolve them with magnetic stirring at 500~600rpm/min, stop heating after Soy lecithin is fully dissolved, add 0.01wt%-0.03wt% Palmitoyl pentapeptide-4, and dissolve it also to obtain the oil phase.

According to some embodiments of the invention, the aqueous phase preparation method in said Step 1) includes: weigh 0.25wt%-1.25wt% Polysorbate 80, 30.00wt%-70.00wt% anti-wrinkle composition, and 0.10wt%-0.20wt% Dendrobium Officinale stem extract, dissolve them in aqua, and in particular, dissolve them by ultrasonic into a homogeneous system to obtain the aqueous phase.

In the invention, the preparation method of Dendrobium Officinale stem extract includes: mixing and extraction of Dendrobium Officinale stem and aqua, filtering, membrane separation, drying, crushing, and sterilization.

In the invention, the CAS No. of Caprylic/Capric triglyceride is 73398-61-5 and that of said Arginine/Lysine polypeptide is 936616-33-0.

Application of the anti-aging composition as claimed in the first aspect of the invention and/or the anti-aging composition prepared in the second aspect of the invention in the field of cosmetics and/or skin care products.

According to the invention, the application of said anti-aging composition in cosmetics and/or skin care products with the efficacy of repairing the skin tissue morphology.

According to the present invention, the application of the anti-aging composition in cosmetics and/or skin care products with the efficacy of accelerating the renewal of collagenous fibers.

According to the invention, the application of the anti-aging composition in cosmetics and/or skin care products with the efficacy of promoting the generation of elastic fibers.

According to the present invention, the application of the anti-aging composition in cosmetics and/or skin care products with the efficacy of anti-aging.

According to the invention, the application of said anti-aging composition in cosmetics and/or skin care products with the efficacy of skin tightening.

Beneficial Effects of the Invention

The invention of anti-aging composition is composed of Soy lecithin, Palmitoyl

pentapeptide-4, Arginine/Lysine polypeptide, Dendrobium Officinale stem extract, Glycerin, Caprylic/Capric triglyceride, Propylene glycol, and Polysorbate 80, which is prepared by high-speed dispersion processes such as high-speed shear and high-pressure homogenization and obtains deformable flexible nano-ethosomes by replacing cholesterol with high-concentration low-chain ethosomes (such as Propylene glycol and Glycerin). The composition has good stability, a high transdermal penetration rate, and good transdermal absorption.

The experimental results show that the invention of anti-aging composition can repair the skin tissue morphology, accelerate the renewal of elastic fibers, promote the generation of collagenous fibers, and have the efficacy of anti-aging and skin tightening.

Brief Description of the Drawings

Drawing 1 shows the influence of the amount of anti-wrinkle composition on the particle size and zeta potential of the anti-aging composition;

Drawing 2 shows the influence of the amount of Soy lecithin on the particle size and zeta potential of the anti-aging composition;

Drawing 3 shows the influence of the amount of Glycerin on the particle size and zeta potential of the anti-aging composition;

Drawing 4 shows the influence of the amount of Caprylic/Capric triglyceride on the particle size and zeta potential of the anti-aging composition;

Drawing 5 shows the influence of the amount of Propylene glycol on the

particle size and zeta potential of the anti-aging composition;

Drawing 6 shows the influence of the amount of Polysorbate 80 on the particle size and zeta potential of the anti-aging composition;

Drawing 7 shows the influence of the shear time on the particle size and zeta potential of the anti-aging composition;

Drawing 8 shows the influence of the shear speed on the particle size and zeta potential of the anti-aging composition;

Drawing 9 shows the influence of the homogenization pressure on the particle size and zeta potential of the anti-aging composition;

Drawing 10 shows the influence of homogenization times on the particle size and zeta potential of the anti-aging composition;

Drawing 11 shows the interaction between the amount of anti-wrinkle composition and the amount of Soy licithin;

Drawing 12 shows the interaction between the amount of anti-wrinkle composition and the amount of Propylene glycol;

Drawing 13 shows the interaction between the amount of Dendrobium Officinale extract and the amount of Propylene glycol;

Drawing 14 shows the fluorescence image (200X) obtained by laser confocal photographing of anti-aging composition in Example 30 and Comparative Example 1;

Drawing 15 shows the slide scanning fluorescence image of Example 30 and Comparative Example 1;

Drawing 16 shows the slide scanning bright field superimposed image of

Example 30 and Comparative Example 1;

Drawing 17 shows the picture of collagenous fibers;

Drawing 18 shows the test results of collagenous fiber content;

Drawing 19 shows the picture of skin tissue morphology;

Drawing 20 shows the picture of elastic fibers;

Drawing 21 shows the test results of elastic fiber content;

Drawing 22 shows the picture of elastin;

Drawing 23 shows the test results of elastin content.

Detailed Description of the Preferred Embodiments

The invention will be described in detail below in combination with the drawings and examples, but the invention is not limited by the following examples.

Unless otherwise specified, the experimental methods described in the following examples are all conventional methods; unless otherwise specified, the experimental materials and reagents can be obtained commercially.

The raw materials used in the invention are shown in Table 1.

Table 1 Raw Materials and Sources

Raw material	Source
Soy lecithin	Tywei Pharmaceutical
Glycerin	Emery
Propylene glycol	SK

Caprylic/Capric triglyceride (CAS No.: 73398-61-5)	Croda Chemical (Shanghai) Co., Ltd.
Polysorbate 80	Tianjin Damao
Anti-wrinkle composition	Activen SA
Palmitoyl pentapeptide-4	Dongguan Huagong Chuangwei Biotechnology Co., Ltd.
Dendrobium Officinale stem extract	Nutri-Woods Bio-tech (Beijing) Co., Ltd.

The information on the instruments/equipment used in the invention is shown in Table 2.

Table 2 Information of Instruments/Equipment

Instrument/Equipment	Manufacturer
Heating magnetic stirrer	IKA
High-speed shear disperser	IKA
Low-temperature ultra-high pressure cell crusher	Guangzhou Juneng Nano & Bio Technology
Ultrasonic cleaner	GT SONIC
High-performance liquid chromatography (HPLC)	SHIMADZU
Low-temperature high-speed centrifuge	Sigma
Dynamic light scattering	Malvern

Examples 1-5

Table 3 Components and Proportions of Anti-aging Composition in Examples 1-5

Sample	Soy lecithin (wt%)	Glyceryl triglyceride (wt%)	Propylene glycol (wt%)	Polysorbate 80 (wt%)	Arginine/Lysine polypeptide (wt%)	pentapeptide composition (wt%)	Officinale stem extract (wt%)	Aqua (wt%)
Example 1	1.00	5.00	1.00	20.00	0.50	0.016	30.0	0.20	To100
Example 2	1.00	5.00	1.00	20.00	0.50	0.016	40.0	0.20	To100
Example 3	1.00	5.00	1.00	20.00	0.50	0.016	50.0	0.20	To100
Example 4	1.00	5.00	1.00	20.00	0.50	0.016	60.0	0.20	To100
Example 5	1.00	5.00	1.00	20.00	0.50	0.016	70.0	0.20	To100

By the weight of the anti-wrinkle composition, said anti-wrinkle composition is composed of 0.0024wt% Arginine/Lysine polypeptide and Aqua To100.

Examples 6-9

Table 4 Components and Proportions of Anti-aging Composition in Examples 6-9

Sample	Soy lecithin (wt%)	Glyceryl triglyceride (wt%)	Propylene glycol (wt%)	Polysorbate 80 (wt%)	Arginine/Lysine polypeptide (wt%)	Chrysanthemum indicum extract (wt%)	Officinale stem extract (wt%)	Aqua
Example 6	0.50	5.00	1.00	20.00	0.50	0.016	50.0	0.20	To100
Example 7	1.50	5.00	1.00	20.00	0.50	0.016	50.0	0.20	To100
Example 8	2.00	5.00	1.00	20.00	0.50	0.016	50.0	0.20	To100
Example 9	2.50	5.00	1.00	20.00	0.50	0.016	50.0	0.20	To100

By the weight of the anti-wrinkle composition, said anti-wrinkle composition is composed of 0.0024wt% Arginine/Lysine polypeptide and Aqua To100.

Examples 10-13

Table 5 Components and Proportions of Anti-aging Composition in Examples 10-13

Sample	Soy lecithin (wt%)	Glyceryl triglyceride (wt%)	Propylene glycol (wt%)	Polysorbate 80 (wt%)	Arginine/Lysine polypeptide (wt%)	Chrysanthemum indicum extract (wt%)	Officinale stem extract (wt%)	Aqua
Example 10	1.00	2.50	1.00	20.00	0.50	0.016	50.0	0.20	To100
Example	1.00	7.50	1.00	20.0	0.50	0.016	50.0	0.20	To10

11				0					0
Example 12	1.00	10.00	1.00	20.00	0.50	0.016	50.0	0.20	To100
Example 13	1.00	12.50	1.00	20.00	0.50	0.016	50.0	0.20	To100

By the weight of the anti-wrinkle composition, said anti-wrinkle composition is composed of 0.0024wt% Arginine/Lysine polypeptide and Aqua To100.

Examples 14-17

Table 6 Components and Proportions of Anti-aging Composition in Examples 14-17

Sample	Soy lecithin (wt%)	Glyceryl (wt%)	triglyceride ric	glycol (wt%) Propylene	80 (wt%) Polysorbate	pentapeptide	composition	Officinale stem extract	Aqua
Example 14	1.00	5.00	1.00	15.00	0.50	0.016	50.0	0.20	To100
Example 15	1.00	5.00	1.00	25.00	0.50	0.016	50.0	0.20	To100
Example 16	1.00	5.00	1.00	30.00	0.50	0.016	50.0	0.20	To100
Example 17	1.00	5.00	1.00	35.00	0.50	0.016	50.0	0.20	To100

By the weight of the anti-wrinkle composition, said anti-wrinkle composition is

composed of 0.0024wt% Arginine/Lysine polypeptide and Aqua To100.

Examples 18-21

Table 7 Components and Proportions of Anti-aging Composition in Examples 18-21

Sample	Soy lecithin (wt%)	Glyceryl triglyceride (wt%)	Propylene glycol (wt%)	Polysorbate 80 (wt%)	pentapeptide (wt%)	composition (wt%)	Officinale stem extract (wt%)	Aqua
Example 18	1.00	5.00	1.00	25.00	0.25	0.016	50.0	0.20	To100
Example 19	1.00	5.00	1.00	25.00	0.75	0.016	50.0	0.20	To100
Example 20	1.00	5.00	1.00	25.00	1.00	0.016	50.0	0.20	To100
Example 21	1.00	5.00	1.00	25.00	1.25	0.016	50.0	0.20	To100

By the weight of the anti-wrinkle composition, said anti-wrinkle composition is composed of 0.0024wt% Arginine/Lysine polypeptide and Aqua To100.

The preparation method of anti-aging composition in Examples 1-21 is shown as follows:

1) According to the anti-aging composition components and proportions as shown in Tables 3-7, weigh Soy lecithin, Glycerin, Caprylic/Capric triglyceride,

and Propylene glycol, dissolve them under 50°C and at 600rpm/min with magnetic stirring, stop heating after Soy lecithin is fully dissolved, add Palmitoyl pentapeptide-4, and dissolve it also to obtain the oil phase; weigh Polysorbate 80, said anti-wrinkle composition, and Dendrobium Officinale stem extract, and dissolve them by ultrasonic into a homogeneous system to obtain the aqueous phase;

2) Add the oil phase to the aqueous phase for hydration for 20min to obtain a mixture of aqueous and oil phases;

3) Carry out high-speed shear at the speed of 9,000rpm for 5min on the mixture of aqueous and oil phases to obtain crude ethosomes;

4) Carry out 5 times of high-pressure homogenization under the pressure of 800bar on crude ethosomes to obtain the anti-aging composition.

Examples 22-30

Table 8 Preparation Process Parameters of Anti-aging Composition in Examples 22-30

Sample	Homogenization Pressure (bar)	Homogenization Times (n)	Shear Speed (rpm)	Shear Time (min)
Example 22	600	3	7000	5
Example 23	600	4	8000	5
Example 24	600	5	9000	5
Example 25	700	3	7000	5

Example 26	700	4	8000	5
Example 27	700	5	9000	5
Example 28	800	3	7000	5
Example 29	800	4	8000	5
Example 30	800	5	9000	5

The preparation method of anti-aging composition in Examples 22-30 is shown as follows:

1) Weigh 1.00wt% Soy lecithin, 5.00wt% Glycerin, 1.00wt% Caprylic/Capric triglyceride, and 25.00wt% Propylene glycol, dissolve them under 50°C and at 600rpm/min with magnetic stirring, stop heating after Soy lecithin is fully dissolved, add 0.016wt% Palmitoyl pentapeptide-4, and dissolve it also to obtain the oil phase for standby; weigh 0.50wt% Polysorbate 80, 50.0wt% anti-wrinkle composition, and 0.20wt% Dendrobium Officinale stem extract powder, dissolve them in deionized water and dissolve them in aqua to obtain the aqueous phase for standby;

2) Add the oil phase to the aqueous phase for hydration for 20min to obtain a mixture of aqueous and oil phases;

3) Carry out high-speed shear on the mixture of aqueous and oil phases to obtain crude ethosomes;

4) Carry out high-pressure homogenization on crude ethosomes to obtain the anti-aging composition.

By the weight of the anti-wrinkle composition, said anti-wrinkle composition is

composed of 0.0024wt% Arginine/Lysine polypeptide and Aqua To100.

The high-speed shear speed, shear time, high-pressure homogenization pressure, and homogenization times of the anti-aging composition in Examples 22-30 are shown in Table 8.

Comparative Example 1

Weigh 1.00wt% Soy lecithin, 5.00wt% Glycerin, 1.00wt% Caprylic/Capric triglyceride, and 25.00wt% Propylene glycol, dissolve them under 50°C and at 600rpm/min with magnetic stirring, stop heating after Soy lecithin is fully dissolved, add 0.016wt% Palmitoyl pentapeptide-4, and dissolve it also to obtain the oil phase; weigh 0.50wt% Polysorbate 80, 50.0wt% anti-wrinkle composition, and 0.20wt% Dendrobium Officinale stem extract powder, dissolve them in deionized water and dissolve them in aqua to obtain the aqueous phase; add the oil phase to the aqueous phase for hydration for 120min to obtain the anti-aging composition of Comparative Example 1.

By the weight of the anti-wrinkle composition, said anti-wrinkle composition is composed of 0.0024wt% Arginine/Lysine polypeptide and Aqua To100.

Test Example 1: Determination of Average Particle Size and Zeta Potential

The zeta potential, average particle size, and particle size distribution of deionized water are measured by using Malvern dynamic light scattering (DLS) at 25°C at 1:1000 dilution of anti-aging composition. Each sample is measured three times in parallel.

According to the scoring method of the hundred score system, the linear interpolation method is used to give the scores of the average particle size, and the samples with different average particle sizes are given different scores. The average particle size is negatively correlated with skin penetration and the average particle size scoring algorithm is shown in the following formula:

$$\text{Average Particle Size Score} = \left\{9 \times \frac{H_{max} - H}{H_{max} - H_{min}} + 1\right\} \times 10$$

Where, H_{max} - Maximum average particle size of sample; H- Average particle size of sample; H_{min} - Minimum average particle size of sample.

1. Test Result of Determination of Average Particle Size and Zeta Potential of the Anti-aging Composition

According to Drawing 1, the amount of anti-wrinkle composition has a great influence on the average particle size of the anti-aging composition, and with the increase in the amount, the average particle size tends to decrease first and then increase, and the absolute value of the zeta potential of the anti-aging composition also features this trend. When the amount of anti-wrinkle composition is 50.0wt%, the average particle size of the anti-aging composition is the minimum (100.32nm), and the zeta potential of anti-aging composition is -39.51mV. Therefore, the amount of 50wt% of anti-wrinkle composition is selected as a preferred amount in the anti-aging composition.

According to Drawing 2, the amount of Soy lecithin has a significant influence on the average particle size of the anti-aging composition, and with the increase

in the amount, the average particle size tends to decrease first and then increase, and the absolute value of the zeta potential of the anti-aging composition is also larger than 40mV, which shows that the samples prepared under said formulation are relatively stable. When the amount of Soy lecithin is 1wt%, the average particle size of the anti-aging composition is the minimum (97.38nm) and the PDI is 0.17. Therefore, the amount of 1wt% of Soy lecithin is selected as a preferred amount in the anti-aging composition.

According to Drawing 3, the amount of Glycin has a significant influence on the average particle size of the anti-aging composition, and with the increase in the amount, the average particle size tends to decrease first and then increase, and then continue to slow down. However, the absolute value of zeta potential tends to increase first and then decrease. When the amount of Glycerin is 2.5wt%-5wt%, the average particle size of the anti-aging composition decreases to the minimum (101.7nm) and the PDI is 0.124, and it belongs to a well-dispersed system, and the zeta potential value of the anti-aging composition is -53.67mV. With the further increase in the amount of Glycin, the average particle size of the anti-aging composition increases, and the absolute value of zeta potential decreases. In consideration of the average particle size of the anti-aging composition and the absolute value of zeta potential, 5wt% will be selected as a preferred amount of Glycin.

According to Drawing 4, the amount of Caprylic/Capric triglyceride has a significant influence on the average particle size of the anti-aging composition, and with the increase in the amount, the average particle size tends to decrease first and then increase, and then slow down. However, the absolute value of zeta

potential tends to increase first and then decrease. When the amount of Caprylic/Capric triglyceride is 0.25wt%-1wt%, the average particle size of the anti-aging composition decreases and the PDI is less than 0.3 with the increase in the amount of Caprylic/Capric triglyceride, and it belongs to a well-dispersed system and the absolute value of zeta potential of anti-aging composition increases. At this time, the average particle size of ethosomes is 100.7nm, the PDI is 0.202, and the zeta potential is -48.7mV. When the amount of Caprylic/Capric triglyceride is 1wt% - 1.25wt%, with the increase in the amount of Caprylic/Capric triglyceride, the average particle size of the anti-aging composition increases significantly, and the absolute value of zeta potential of anti-aging composition also decreases. In consideration of the average particle size of the anti-aging composition and the absolute value of zeta potential, 1wt% will be selected as a preferred amount of Caprylic/Capric triglyceride.

According to Drawing 5, the amount of Propylene glycol has a significant influence on the average particle size of the anti-aging composition, and with the increase in the amount, the average particle size tends to decrease first and then increase, and then slow down. However, the absolute value of zeta potential tends to increase first and then decrease. When the amount of Propylene glycol is 15wt%-25wt%, with the increase in the amount of Propylene glycol, the average particle size of the anti-aging composition decreases continuously to 82.44nm. With the further increase of Propylene glycol, the average particle size of the anti-aging composition increases significantly, and the PDI is all around 0.2, indicating that the sample systems obtained under various formulations belong to centralized and uniform

dispersion systems. In consideration of better skin penetration with small particle size, 25wt% will be selected as a preferred amount of Propylene glycol.

According to Drawing 6, the amount of Polysorbate 80 has a significant influence on the average particle size of the anti-aging composition, and with the increase in the amount, the average particle size tends to decrease first and then increase, and then slow down. However, the absolute value of zeta potential tends to increase first and then decrease. When the amount of Polysorbate 80 is 0.25wt%-1wt%, with the increase in the amount of Polysorbate 80, the average particle size of the anti-aging composition decreases first and then increases, but the overall change is not large. When the amount of Polysorbate 80 is 0.5wt%, the particle size is the minimum (83.79nm), the zeta potential is -33.0mV, and the PDI is 0.201. With the future increase in the amount of Polysorbate 80 to 1.25wt%, the average particle size of the anti-aging composition significantly increases to 158.67nm. In consideration of the average particle size of the anti-aging composition and the absolute value of zeta potential, 0.5wt% is selected as a preferred amount of Polysorbate 80.

According to Drawings 7 and 8, with the increase in the time of high-speed shear, the average particle size of the anti-aging composition continues to decrease to maintain equilibrium, and the absolute value of zeta potential first increases and then changes little. The average particle size of ethosomes prepared with the anti-aging composition is 94.1nm when the shear time is 3min. With the increase in the shear time, the average particle size of the anti-aging composition does not change much; and the absolute value of zeta potential of anti-aging composition also features this trend. In consideration of better skin

penetration with small particle size, production efficiency, etc., the shear time of 3min will be selected as a preferred process parameter for preparing the anti-aging composition. With the increase in the shear speed, when the shear speed is 8,000rpm, the average particle size of the anti-aging composition decreases and then remains unchanged, and the average particle size at this time is 90.83nm. In consideration of the production cost, the shear speed of 8,000rpm is selected as a preferred process parameter for preparing the anti-aging composition.

According to Drawings 9 and 10, with the increase in the high-pressure homogenization pressure, the average particle size of the anti-aging composition decreases first and then changes little, and the absolute value of zeta potential increases first and then decreases. As for the average particle size of the anti-aging composition, when the homogenization pressure is 700bar, the average particle size of prepared ethosomes is 82.5nm and the absolute value of zeta potential of anti-aging composition is the maximum. In consideration of better skin penetration with small particle size, instrument energy consumption, etc., 700bar will be selected as a preferred process parameter for preparing the anti-aging composition. According to the graph data of the influence of different homogenization times on the particle size and zeta potential of ethosomes, after four times of homogenization, the average particle size of the anti-aging composition is 83.4nm. With the increase in the homogenization times, the average particle size of the anti-aging composition changes little. In consideration of the production cost, four times of homogenization are selected as a preferred process parameter for preparing the anti-aging composition.

Test Example 2: Orthogonal Test

According to the single factor test results, the factors with a large average particle size of the anti-aging composition, i.e. high-pressure homogenization pressure of factor A_{-1}, homogenization times of factor B_{-1}, and high-speed shear speed of factor C_{-1}, are selected for orthogonal test according to three factors and three levels. The orthogonal test design is shown in Table 9.

Table 9 Optimization Factors and Levels of Process Parameters of Anti-aging Composition

Factor Level	Homogenization Pressure (bar)	Homogenization Times (n)	Shear Speed (rpm)
	A_{-1}	B_{-1}	C_{-1}
1	600	3	7,000
2	700	4	8,000
3	800	5	9,000

Test Example 3: Response Surface Test

According to the single factor test results, materials with a large influence on the average particle size of the anti-aging composition are selected, i.e., the amount of anti-wrinkle composition as Factor A, the amount of Soy lecithin as Factor B, and the amount of Propylene glycol as Factor C are selected as the response surface factors, 17 groups of tests are designed with the Box-Behnken, the particle size scores are taken as the response value in the test results, and the

design of response surface test is shown in Table 10.

Table 10 Design of Response Surface Test

Group	Factor		
	A	B	C
1	40	0.75	25
2	40	1.25	25
3	60	0.75	25
4	50	1	25
5	40	1	20
6	40	1	30
7	50	1	25
8	50	1.25	20
9	50	0.75	20
10	60	1	20
11	60	1	30
12	50	1	25
13	60	1.25	25
14	50	1.25	30
15	50	1	25
16	50	1	25
17	50	0.75	30

2. Orthogonal Results and Analysis of Preparation Method of Anti-aging Composition

Table 11 Orthogonal Test Design and Results of Process Parameter Optimization of Anti-aging Composition

No.	Homogenization Pressure	Homogenization Times	High-speed Shear Speed	Particle Size (nm)	Particle Size Score
	A_{-1}	B_{-1}	C_{-1}		
1	1	1	1	182.7	10.00
2	1	2	2	168	22.30
3	1	3	3	163.2	26.31
4	2	1	2	135.3	49.65
5	2	2	3	112.7	68.55
6	2	3	1	105.1	74.91
7	3	1	3	87.56	89.58
8	3	2	1	77.63	97.88
9	3	3	2	75.1	100.00
K1	19.54	49.74	60.93		
K2	64.37	62.91	57.32		

K3	95.82	67.07	61.48		
R	76.28	17.33	4.16		
Optimal	A3	B3	C3		

The test is arranged according to the table of the orthogonal test of three factors and three levels and the results are shown in Table 11. The results show that the preferred process range of anti-aging composition is high-pressure homogenization pressure of 600-800bar, homogenization times of 3-5, and high-speed shear speed of 7,000-9,000rpm. The influence degree of each factor on the comprehensive index is $A_{-1} > B_{-1} > C_{-1}$, that is, within the selected range of the test design, the homogenization pressure has the largest impact on the comprehensive index, the homogenization times is the second, and the shear speed has the smallest impact. The larger the index value, the better. Therefore, the orthogonal optimal formulation is $A_{-1}3B_{-1}3C_{-1}3$, that is, the homogenization pressure of 800bar, the homogenization times of 5, and the shear speed of 9,000rpm.

3. Design and Results of Response Surface Test of Component Amounts of Anti-aging Composition

Table 12 Design and Results of Response Surface Test

Group	Factor			Particle Size (nm)	Score
	A	B	C		

1	40	0.75	25	72.36	98.35
2	40	1.25	25	78.43	87.23
3	60	0.75	25	76.31	91.12
4	50	1	25	71.46	100.00
5	40	1	20	73.94	95.46
6	40	1	30	93.5	59.63
7	50	1	25	72.41	98.26
8	50	1.25	20	88.69	68.44
9	50	0.75	20	81.07	82.40
10	60	1	20	72.18	98.68
11	60	1	30	112.6	24.65
12	50	1	25	72.05	98.92
13	60	1.25	25	88.24	69.27
14	50	1.25	30	120.6	10.00
15	50	1	25	72.48	98.13
16	50	1	25	71.98	99.05
17	50	0.75	30	111.8	26.12

The data are regression fitted and the analysis results are shown in Table 12. By regression fitting the three factors of the amount of anti-wrinkle composition, the amount of Soy lecithin, and the amount of Propylene glycol, the quadratic regression equation is obtained as follows: Y = 98.87 - 7.12*A - 7.88*B -

28.07*C - 2.68*A*B - 9.55*A*C - 0.54*B*C + 5.24*A^2 - 17.62*B^2 - 34.51*C^2. The significance of the coefficient and the interaction strength of the combined factors are determined by the *P*-value, and the smaller the *P*-value is, the more significant it is. According to the variance analysis, $P<0.0001$ means that it is extremely significant in the regression model, and $P>0.05$ means that it is not significant and that the model fits the test data well. The determination coefficient R^2 is 0.9992, which indicates that the model has a good fitting and a small experimental error. The correction determination coefficient R^2adj of the regression model is 0.9981, which indicates that the model can explain 99.81% of the change in response value, and only 0.19% of the change cannot be explained by the model, again proving the effectiveness of the model. The variance analysis of the model shows that the amount of anti-wrinkle composition (A), the amount of Soy lecithin (B), the amount of Propylene glycol (C), the interaction between the amount of anti-wrinkle composition and the amount of Soy lecithin (AB), the interaction between the amount of anti-wrinkle composition and the amount of Propylene glycol (AC), and the quadratic term of each factor have significant influences on the response value. The order of the factors influencing the comprehensive score of the product is obtained as follows: (C) amount of Propylene glycol > (B) amount of Soy lecithin > (A) amount of anti-wrinkle composition.

Table 13 Variance Analysis of Regression Model

Regression	Square	Degree	Mean	*F*-value	*P*-	Significanc

Item	Sum	of Freedom	Square		value	e
Model	14,234.74	9	1,581.64	952.5	< 0.0001	**
A (Anti-wrinkle composition)	405.41	1	405.41	244.15	< 0.0001	**
B (Soy lecithin)	496.91	1	496.91	299.25	< 0.0001	**
C (Propylene glycol)	6,304.52	1	6,304.52	3,796.74	< 0.0001	**
AB	28.78	1	28.78	17.33	0.0042	**
AC	364.81	1	364.81	219.7	< 0.0001	**
BC	1.17	1	1.17	0.7	0.4297	NS

A2	115.73	1	115.73	69.7	< 0.0001	**
B2	1,307.55	1	1,307.55	787.44	< 0.0001	**
C2	5,014.41	1	5,014.41	3,019.81	< 0.0001	**
Residual error	11.62	7	1.66			
Lack of fit	9.39	3	3.13	5.61	0.0645	NS
Error item	2.23	4	0.56	/	/	/
Total variance	14,246.36	16	/	/	/	/
R^2	0.9992	/	/	/	/	/
R^2adj	0.9981	/	/	/	/	/

Note: ** extremely significant difference, $P<0.01$; * significant difference ($P<0.05$); no significant difference (NS), $P>0.05$

The response surface and contour graph of the interaction among the three factors of the amount of anti-wrinkle composition, the amount of Soy licithin, and the amount of Propylene glycol are shown in Drawings 11-13. The results

show that the preferred formulation range of the anti-aging composition is the amount of anti-wrinkle composition of 40wt%-60wt%, the amount of Soy licithin of 0.75-1.25wt%, and the amount of Propylene glycol of 20wt%-30wt%. According to the response surface graph and contour graph, the influence of the interaction of the two test factors on the response value can be intuitively reflected. If the interaction of the two factors has a greater influence on the response value, the response surface graph will be steeper and the contour line will be elliptical. If the interaction of the two factors has no significant influence on the response value, the contour line will be circular. In Figure (a) and Figure (c), the response surface is convex to some extent and the contour graph is elliptical, indicating that the interaction between the amount of Arginine/Lysine polypeptide and the amount of Soy licithin is significant, and the interaction between the amount of anti-wrinkle composition and the amount of Propylene glycol is significant.

The Box-Behnken response surface test predicts the optimal formulation parameters as follows: the amount of anti-wrinkle composition of 50wt%, the amount of Soy licithin of 1wt%, and the amount of Propylene glycol of 25wt%.

Test Example 4: Transdermal Penetration Rate

1. Samples to be tested: Example 30 and Comparative Example 1

2. Test purpose: To evaluate the transdermal absorption ability of the product through excised skin.

3. System and management

1) Experimental animal:

2) Name: SD rat (male, 7 weeks old), grade: SPF

3) Source: Provided by Zhuhai Bestest Bio-Tech Co., Ltd., production license No.: SCXK(Y) 2016-004

4) Reagent

Table 14 Reagent Types

Name	Brand
PBS	Solarbio Biosharp
Normal saline	Henan Kelun Pharmaceutical Co., Ltd.
4% paraformaldehyde	Biosharp

5) Instruments and equipment

Table 15 Instruments and Equipment

Name	Brand	Model
Laser scanning confocal microscope	NIKON	NIKON Eclipse Ti
Digital slide scanner	3D HISTECH	Pannoramic DESK
Freezing microtome	THERMO	CRYOSTAR NX50

4. Test steps

Administration method: Shave off the SD rat qualified in quarantine with a shaver, cut off the back skin, and wash it with normal saline. Remove the

subcutaneous fat, cut it into the skin with a diameter/side length of about 2cm, drop 0.3mL of each prepared marker onto the nonwoven fabric with the same size, and fix it on the shaved rat skin. After each sample is applied for 15min, 30min, 60min, and 120min respectively, suck up the skin surface liquid and place it in the 4% paraformaldehyde fixation solution.

5. Test indexes

1) Carry out scanning and analysis by laser scanning confocal microscope - label the samples with FITC labels and observe with a single green fluorescent channel, and observe by scanning with the wavelength of 530nm and above after the excitation wavelength is 488nm.

2) Slide scanning: Use a digital scanner to scan the slices to obtain fluorescence scanning images and white light (bright field) scanning images.

6. Result analysis

The confocal pictures are analyzed and fluorescence intensity is calculated: fluorescence intensity = fluorescence value / average fluorescence area. The scanning image processing software superimposes the fluorescence image and the bright field scanning image. The fluorescence penetration depth is analyzed according to the bright field superposition image.

7. Data statistics

All data are expressed in (mean ± SD) and analyzed by SPSS software. If the variance of measurement data is uniform or the variance of the data is uniform after conversion, a one-factor analysis of variance shall be used. If the variance of the data is still not uniform after conversion, the rank sum test shall be used

for statistical analysis. Test level α=0.05.

The fluorescence intensity analysis of skin images of anti-aging composition is shown in Table 16 and the fluorescence image obtained from laser scanning confocal microscope scanning is shown in Drawing 14.

Table 16 Fluorescence Intensity Analysis

Sample to Be Tested	Fluorescence Intensity (AU)			
	15min	30min	60min	120min
Example 30	84.530±0.994 *	46.852±0.167 *	44.848±9.687	78.461±5.090 *
Comparative Example 1	41.188±1.040	33.354±1.164	40.242±6.206	38.161±4.073

Note: * means that there is a statistically significant difference between the data and the comparative sample, $P<0.05$.

The results show that the fluorescence intensity of Example 30 Anti-aging Composition is larger than that of the comparative sample (non-lipidated) during the same duration of action after 15min, 30min, and 120min, with a significant difference ($p<0.05$).

In Drawing 15, the fluorescence image obtained from laser scanning confocal microscope scanning shows that the fluorescence intensity of Example 30 anti-aging composition is larger than that of the comparative sample.

The fluorescence penetration depths of the cortex obtained from the slide scanning are shown in Table 17, the fluorescence images are shown in Drawing 15, and the bright field superimposed images are shown in Drawing 16.

Table 17 Fluorescence Penetration Depth Analysis

Sample to Be Tested	Fluorescence Intensity (AU)			
	15min	30min	60min	120min
Example 30	1249.433±64.430	1478.600±56.591	1342.267±228.441	1338.533±170.303
Comparative Example 1	1374.733±118.635	1444.867±321.613	1240.200±54.631	1371.933±38.209

Note: * means that there is a statistically significant difference between the data and the comparative sample, $P<0.05$.

As shown in the slice scanning fluorescence images and the slice scanning bright field superimposed images, there is no significant difference in penetration depth between Example 30 anti-aging composition and the comparative example.

Examples 31-32

Table 18 Formulation of Anti-aging Face Cream

Group Phase	Raw Material	INCI Name	Example 31	Example 32

			Amount (wt%)	
A	Aqua	Aqua	To100	To100
	KELTROL CG-T	Xanthan gum	0.08	0.08
	Glycerin	Glycerin	2.00	2.00
B	A170	Glyceryl stearate/PEG-100 Stearate	2.50	2.50
	Mixed ethosomes	Cetearyl alcohol	1.00	1.00
	PES	Pentaerythrityl distearate	1.50	1.50
	Polysynlane	Hydrogenated polyisobutene	3.00	3.00
	GTCC	Caprylic/Capric triglyceride	3.00	3.00
	DM100	Polydimethylsiloxane	2.00	2.00
	ISIS	Isooctadecyl isooctadecanoate	3.00	3.00
C	MTI	Methylisothiazolinone/ Iodopropynyl butylcarbamate	0.05	0.05
	PEHG	Phenoxyethanol/Ethylhexylglycerin	0.05	0.05
D	Example 30 Anti-aging Composition	/	5.00	10.00

The preparation process of Example 31 Anti-aging Face Cream is shown as follows:

1. First premix xanthan gum and glycerin in Group A, add them into deionized water, stir, and heat to 85°C, keep stirring for 30min until xanthan gum is completely dispersed and dissolved, and keep it on standby;

2. Mix the materials of Group B, heat them to 85°C to melt the solid, and keep it on standby;

3. Quickly add Group B to Group A, stir and mix evenly, and homogenize for 5min until complete emulsification;

4. Cool down to 40°C, add Group C and Group D, stir and mix evenly;

5. Pass the membrane and discharge them.

The preparation process of Example 32 Anti-aging Face Cream is shown as follows:

1. First premix xanthan gum and glycerin in Group A, add them into deionized water, stir, and heat to 80°C, keep stirring for 30min until xanthan gum is completely dispersed and dissolved, and keep it on standby;

2. Mix the materials of Group B, heat them to 80°C to melt the solid, and keep it on standby;

3. Quickly add Group B to Group A, stir and mix evenly, and homogenize for 3min until complete emulsification;

4. Cool down to 40°C, add Group C and Group D, stir and mix evenly;

5. Pass the membrane and discharge them.

Comparative Examples 1-2

Table 19 Formulation of Anti-aging Face Cream

Group Phase	Raw Material	INCI Name	Example 31	Example 32
			Amount (wt%)	
A	Aqua	Aqua	To100	To100
	KELTROL CG-T	Xanthan gum	0.08	0.08
	Glycerin	Glycerin	2.00	2.00
B	A170	Glyceryl stearate/PEG-100 Stearate	2.50	2.50
	Mixed ethosomes	Cetearyl alcohol	1.00	1.00
	PES	Pentaerythrityl distearate	1.50	1.50
	Polysynlane	Hydrogenated polyisobutene	3.00	3.00
	GTCC	Caprylic/Capric triglyceride	3.00	3.00
	DM100	Polydimethylsiloxane	2.00	2.00
	ISIS	Isooctadecyl isooctadecanoate	3.00	3.00
C	MTI	Methylisothiazolinone/ Iodopropynyl butylcarbamate	0.05	0.05

	PEHG	Phenoxyethanol/Ethylhexylglycerin	0.50	0.50
D	Retinyl palmitate	Retinyl palmitate	0.20	/

The preparation process of the anti-aging face creams in Comparative Examples 1-2 is the same as that of Example 31.

Test Example 5: Tightening and Anti-wrinkle Test

1. Test method

1.1 Test system

The excised skin tissue used in this test is obtained from Guangdong BioCell Biotechnology Co., Ltd.

1.2 Main reagents

Excised skin tissue culture medium (FSK4, BioCell Biotechnology), vitamin C (VC, Sigma), vitamin E (VE, Sigma), paraformaldehyde (Sinopharm), and PBS (BOSTER).

1.3 Main equipment

CO_2 incubator (Thermo, 150i), ultra-clean bench (Suzhou Antai, SW-CJ-1F), fluorescence microscope (Leica, DM2500), and upright metallurgical microscope (Olympus, BX53).

1.4 Test samples

Examples 31-32 and Comparative Examples 1-2

1.5 Test scheme

(1) Configure the sample working fluid according to Table 20 of the test scheme.

Table 20 Sample Working Fluid

Group	Administration Concentration	Induction Condition	Testing Model
BC	/	/	Ex vivo skin tissue
NC	/	30J/cm^2 UVA + 50mJ/cm^2 UVB	
PC (VC+VE)	100μg/mL + 7μg/mL		
Example 31	Stock solution		
Example 32			
Comparative Example 1			
Comparative Example 2			

(2) Tissue processing: Wash the freshly obtained skin tissue by immersing it in 75% alcohol for 30s and then wash three times with sterile PBS buffer solution; after washing, use a disposable sterile skin sampler to cut the skin tissue into small discs of 0.6cm in diameter, place them in culture molds with the epidermis facing up and the dermis facing down, transfer the culture molds into 6-well plates, add 3.7mL of culture solution per well, incubate at 37°C in a 5% CO_2 incubator, and change the solution every day.

(3) Administration: After 2 days of tissue culture in vitro, start UVR irradiation and administration referring to the groups and corresponding treatment conditions in Table 19; the UVR irradiation doses are UVA (30 J/cm^2) and UVB (50 mJ/cm^2) for 4 consecutive days, and replace the culture medium after each irradiation and perform administration. The positive comparative (VC+VE) group is administered sublingually and the sample to be tested is administered superficially. After 4 days of continuous irradiation, the isolated skin tissue is cultured for another 3 days, during which time no UVR stimulation is performed and the only sample is administered.

(4) Testing of tissue morphology, collagenous fiber and elastic fiber: skin tissues at the end of drug administration are fixed in a 4% paraformaldehyde model, embedded, sectioned for H&E staining, Masson staining, and Victoria Blue staining, respectively, and the section results are recovered and photographed by microscope for analysis using Image-Pro®Plus.

(5) Analysis of data results: GraphPad Prism Program software is used for drawing, and t-test is used for statistical analysis between groups. $P<0.05$ means a significant difference and $P<0.01$ means an extremely significant difference.

2. Test results

2.1 Collagenous fiber test results

Table 21 Test Results of Collagenous Fiber Content

Group	Average Relative Area	SD	*P*-value
BC	1.00	0.04	/
NC	0.33	0.02	0.000##
PC	0.94	0.06	0.000**
Example 31	0.86	0.06	0.000**
Example 32	0.85	0.13	0.002**
Comparative Example 1	0.87	0.07	0.000**
Comparative Example 2	0.44	0.08	0.088

Note: When the t-test method is used for statistical analysis, compared with the BC group, the significance is indicated by #, *P*-value＜0.05 by #, and *P*-value＜0.01 by ##; compared with the NC group, the significance is indicated by *, *P*-value＜0.05 by *, and *P*-value＜0.01 by **.

The test results of collagenous fiber content are shown in Table 21 and Drawings 17-18. Compared with the BC group, the collagenous fiber content of the NC group decreases significantly, which indicates that the stimulation conditions in this test are effective. Compared with the NC group, the collagenous fiber content in the PC group increases significantly, which indicates that the positive comparative test is effective; the collagenous fiber contents in Example 31 and Example 32 are significantly higher than that of the

NC group.

2.2 Tissue morphology test results

The tissue morphology test results are shown in Drawing 19. Compared with the BC group, the living cell layer of the epidermis in the NC group is thinner, and the density of fibroblasts in the dermis decreases, indicating that the UVR stimulation condition is effective. Compared with the NC group, the living cell layer of the epidermis in the PC group is significantly thickened, and the density of fibroblasts in the dermis increases, indicating that the positive comparative test is effective. Compared with the NC group, the living cell layer of the epidermis in the administration group of Example 31, is thickened, and the density of fibroblasts in the dermis of Example 31 increases, indicating that the sample has improved the tissue morphology to a certain extent.

2.3 Elastic fiber test results

Table 22 Test Results of Elastic Fiber Content

Group	Average Relative Area	SD	*P*-value
BC	1.00	0.15	/
NC	0.23	0.03	0.001##
PC	0.76	0.11	0.002**
Example 31	0.79	0.10	0.001**

Comparative Example 1	0.45	0.07	0.009**
Comparative Example 2	0.31	0.11	0.295

Note: When the t-test method is used for statistical analysis, compared with the BC group, the significance is indicated by #, *P*-value＜0.05 by #, and *P*-value＜0.01 by ##; compared with the NC group, the significance is indicated by *, *P*-value＜0.05 by *, and *P*-value＜0.01 by **.

The test results of elastic fiber content are shown in Table 22 and Drawings 20-21. Compared with the BC group, the elastic fiber content of the NC group decreases significantly, which indicates that the stimulation conditions in this test are effective. Compared with the NC group, the elastic fiber content in the PC group increases significantly, which indicates that the positive comparative test is effective; the elastic fiber content in Example 31 is significantly higher than that of the NC group.

2.4 Elastin test results

Table 23 Rest Results of Elastin Content

Group	Average Relative Area	SD	*P*-value
BC	1	0.12	/

NC	0.42	0.05	0.001##
PC	0.71	0.03	0.001**
Example 31	0.63	0.09	0 024**
Comparative Example 1	0.50	0.07	0.144
Comparative Example 2	0.32	0.04	0.058

Note: When the t-test method is used for statistical analysis, compared with the BC group, the significance is indicated by #, *P*-value＜0.05 by #, and *P*-value＜0.01 by ##; compared with the NC group, the significance is indicated by *, *P*-value＜0.05 by *, and *P*-value＜0.01 by **.

Compared with the BC group, the NC group shows a significant decrease in elastin content, indicating the effectiveness of the stimulation conditions. Compared with the NC group, the elastin content in the PC (VC + VE) group increases significantly, indicating the effectiveness of the positive comparative test. Compared with the NC group, the elastin content in Example 31 increases significantly.

Based on the ex vivo skin tissues, an aging model is established by UV induction. Compared with the NC group, in the administration group of Example 31 Anti-aging Face Cream, the anti-aging face cream increases, the tissue morphology is improved to a certain extent, and the collagenous fiber

content increases significantly. It is believed that Example 31 Anti-aging Face Cream can antagonize wrinkles caused by collagen loss in the process of skin aging by increasing the total amount of collagen so as to achieve the anti-wrinkle effect.

Compared with the NC group, in the administration group of Example 31 Anti-aging Face Cream, the elastic fiber content and elastin content increase significantly. It is believed that Example 31 Anti-aging Face Cream has a tightening effect by enhancing elastic fibers and elastin to antagonize the decrease in elasticity during skin aging.

Claims:

1. An anti-aging composition, which is characterized in that, by weight percentage, said anti-aging composition is composed of 30.00wt%-70.00wt% anti-wrinkle composition, 0.50wt%-2.50wt% Soy lecithin, 2.50wt%-12.50wt% Glycerin, 0.25wt%-1.25wt% Caprylic/Capric triglyceride, 15.00wt%-35.00wt% Propylene glycol, 0.25wt%-1.25wt% Polysorbate 80, 0.01wt%-0.03wt% Palmitoyl pentapeptide-4, 0.10wt%-0.20wt% Dendrobium Officinale stem extract, and Aqua To100; and

by the weight of the anti-wrinkle composition, said anti-wrinkle composition is composed of 0.001wt%-0.005wt% Arginine/Lysine polypeptide and Aqua To100.

2. A method for preparing the anti-aging composition as claimed in Claim 1, which is characterized in that, said method comprises the following steps:

1) Preparation of oil phase and aqueous phase, said oil phase includes Soy lecithin, Glycerin, Caprylic/Capric triglyceride, Propylene glycol, and Palmitoyl pentapeptide-4; said aqueous phase includes Polysorbate 80, anti-wrinkle composition, and Dendrobium Officinale stem extract;

2) Add the oil phase to the aqueous phase for hydration to obtain a mixture of aqueous and oil phases;

3) Carry out high-speed shear on the mixture of aqueous and oil phases to obtain crude ethosomes;

4) Carry out high-pressure homogenization on crude alcohols to obtain

ethosomes.

3. The method as claimed in Claim 2, which is characterized in that, the oil phase preparation method in said Step 1) includes: weigh Soy lecithin, Glycerin, Caprylic/Capric triglyceride, and Propylene glycol, dissolve them, add Palmitoyl pentapeptide-4, and dissolve it also to obtain the oil phase; and/or said aqueous phase preparation method includes: weigh Polysorbate 80, said anti-wrinkle composition, and Dendrobium Officinale stem extract, and dissolve them in aqua to obtain the aqueous phase.

4. The method as claimed in Claim 2 or 3, which is characterized in that, the hydration time in said Step 2) is 10-30min.

5. The method as claimed in any of Claims 2-4, which is characterized in that, the high-speed shear speed in said Step 3) is 6,000-10,000rpm and the time of high-speed shear is 1-5min.

6. The method as claimed in any of Claims 2-5, which is characterized in that, the high-pressure homogenization pressure in said Step 4) is 600-1,000bar and the number of high-pressure homogenization is 3-7 times.

7. The method as claimed in any of Claims 2-6, which is characterized in that, said preparation method includes the following steps:

1) Weigh 0.50wt%-2.50wt% Soy lecithin, 2.50wt%-12.50wt% Glycerin, 0.25wt%-1.25wt% Caprylic/Capric triglyceride, and 15.00wt%-35.00wt% Propylene glycol, dissolve them, add 0.01wt%-0.03wt% Palmitoyl pentapeptide-4, and dissolve it also to obtain the oil phase; weigh 0.25wt%-1.25wt% Polysorbate 80, 30.00wt%-70.00wt% anti-wrinkle composition, and

0.10wt%-0.20wt% Dendrobium Officinale stem extract and dissolve them in aqua to obtain the aqueous phase;

2) Add the oil phase to the aqueous phase for hydration for 10-30min to obtain a mixture of aqueous and oil phases;

3) Carry out high-speed shear at the speed of 6,000-10,000rpm for 1-5min on the mixture of aqueous and oil phases to obtain crude ethosomes;

4) Carry out 3-7 times of high-pressure homogenization under the pressure of 600-1,000bar on crude alcohols to obtain ethosomes.

8. Application of the anti-aging composition as claimed in Claim 1 in the field of cosmetics and/or skin care products.

9. The application as claimed in Claim 8, which is characterized in that, said anti-aging composition can repair the skin tissue morphology and/or accelerate the renewal of elastic fibers and/or promote the generation of collagenous fibers and/or resist aging and/or tighten the skin.