COMPOSITION FOR HAIR


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ABSTRACT

It is an object of the present invention to provide a composition for hair which comprises highly safe nanoparticles having high transparency due to the small particle size and high permeability into hair and scalp. The present invention provides a composition for hair which comprises protein nanoparticles containing an active ingredient for hair.
COMPOSITION FOR HAIR

TECHNICAL FIELD

[0001] The present invention relates to a composition for hair, which comprises protein nanoparticles containing an active ingredient for hair.

BACKGROUND ART

[0002] Extensive applications of fine particle materials have been expected for biotechnology. In particular, the application of nanoparticle materials generated based on the advancement of nanotechnology to food, cosmetics, pharmaceutical products, and the like has been actively discussed. In this regard, the results of many studies have been reported.

[0003] For instance, regarding cosmetics, more obvious skin-improving effects have been required in recent years. Manufacturers have been attempting to improve the functionality and usability of their own products and to differentiate their own products from competitive products by applying a variety of technologies such as nanotechnology. In general, the stratum corneum serves as a barrier for the skin. Thus, medicines are unlikely to permeate therethrough into the skin. In order to obtain sufficient skin-improving effects, it is essential to improve the skin permeability of active ingredients. In addition, it is difficult to formulate many active ingredients due to poor preservation stability or tendency to result in skin irritancy, although they are highly effective to the skin. In order to solve the above problems, a variety of fine particle materials have been under development for the improvement of transdermal absorption and preservation stability, reduction of skin irritancy, and the like. Recently, a variety of fine particle materials such as ultrafine emulsions and liposomes have been studied (e.g., Mitsuhiro Nishida, Fragrance Journal, Nov., 17 (2005)).

[0004] With the use of polymeric materials instead of emulsified products or liposomes, it can be expected that remarkable improvement in preservation stability and in vivo particle stability will be achieved due to the structure of such material. However, in most studies, synthetic polymers obtained by, for example, emulsion polymerization are used, so that it is required to obtain safer carriers.

[0005] Further, Hiroki Fukui, Polymer, October, 798(2006) describes the study of reservoir properties of a phospholipid polymer nanoparticle whereby an active ingredient is maintained in hair. However, it is not easy to design and synthesize such self-organized polymer. Thus, it is difficult to commercialize such polymer in terms of cost.

[0006] As an aside, hair is damaged by environmental factors such as ultraviolet irradiation and chlorine contact, chemical factors such as coloring, decoloring, permanent wave, and hair washing with the use of shampoo comprising strong surfactants, and physical factors such as the overuse of dryers at high temperatures.

[0007] Such damage results in unfavorable hair conditions such as loss of cuticles or proteins and hardened, brittle, or split hair.

[0008] Hitherto, many ingredients have been said to be effective for treatment or prevention of hair damage. Such ingredients have been used for protection of hair from ultraviolet rays or dryness, enhancement of hair volume or strength, prevention of hair loss, improvement against hair decrease, and the like. However, the above ingredients are not sufficiently effective.

[0009] Meanwhile, it is also important for a hair growth agent not only to contain an excellent hair growth component but also to have an active ingredient that can be securely delivered to action sites.

[0010] In addition, some compositions for hair contain 50% or more ethanol, and its adverse effects on the scalp are causes for concern. In the field of hair growth agents, hair growth agents generally contain 50% or more ethanol in order to dissolve hydrophobic hair growth components so that adverse effects caused by ethanol are causes for concern. JP Patent Publication (Kokai) No. 2006-176447 A suggests that scalp irritation caused by ethanol can be alleviated by a composition for hair.

DISCLOSURE OF THE INVENTION

[0011] It is an object of the present invention to solve the above problems of the prior art. Specifically, it is an object of the present invention to provide a composition for hair which comprises highly safe nanoparticles having high transparency due to the small particle size and high permeability into hair and scalp, such composition being formulated as a shampoo, a rinse, a hair conditioner, a hair pack, a hair liquid, a hair tonic, a hair spray, or the like.

[0012] As a result of intensive studies in order to achieve the above object, the present inventors demonstrated that a protein nanoparticle containing an active ingredient for hair which were prepared by the inventors are highly safe and have high transparency and favorable permeability into hair and scalp. The present invention has been completed based on the above findings.

[0013] That is, the present invention provides a composition for hair which comprises protein nanoparticles containing an active ingredient for hair.

[0014] Preferably, the composition comprises 0.01% to 50% by weight protein nanoparticles.

[0015] Preferably, the average particle size of protein nanoparticles is 10 to 1000 nm.

[0016] Preferably, the protein nanoparticles contain an active ingredient for hair in a weight that is 0.1% to 100% of the protein weight.

[0017] Preferably, the active ingredient for hair is at least one selected from the group consisting of cosmetic ingredients and pharmaceutical ingredients.

[0018] More preferably, the active ingredient for hair is an ionic substance or a fat-soluble substance.

[0019] Further preferably, the active ingredient for hair is a hair growth agent.

[0020] Preferably, the ethanol content in the composition for hair of the present invention is 20% by weight or less.

[0021] Preferably, the protein is at least one selected from the group consisting of collagen, gelatin, acid-treated gelatin, albumin, ovalbumin, casein, transferrin, globulin, fibrin, fibrin, laminin, fibropectin, and vitronectin.

[0022] Preferably, the protein is subjected to crosslinking treatment during and/or after nanoparticle formation.

[0023] Preferably, an enzyme can be used as a crosslinking agent.

[0024] The enzyme is not particularly limited as long as it has the effect of causing protein crosslinking. However, transglutaminase can be preferably used.
Preferably, the composition for hair of the present invention comprises casein nanoparticles prepared by the following steps (a) to (c):

(a) mixing casein with a basic aqueous medium at pH of from 8 to less than 11;
(b) adding at least one active ingredient for hair to the solution obtained in step (a);
(c) injecting the solution obtained in step (b) into an acidic aqueous medium at pH of 3.5 to 7.5.

Preferably, the composition for the hair of the present invention comprises casein nanoparticles prepared by the following steps (a) to (c):

(a) mixing casein with a basic aqueous medium at pH of from 8 to less than 11;
(b) adding at least one active ingredient for hair to the solution obtained in step (a);
(c) lowering the pH of the solution obtained in step (b) to pH value which is different from the isoelectric point by 1 or more units, while stirring the solution.

The particles containing an active ingredient for hair in the composition for hair of the present invention are nanoparticles, and thus they are highly absorbable. In addition, according to the present invention, since protein nanoparticles are used, there is no need to use chemical crosslinking agents or synthetic surfactants for production of the composition, which is highly safe. Moreover, a hydrophobic active ingredient for hair can be formed in a nanoparticle dispersion. Accordingly, there is no need to add ethanol in large amounts and thus scalp irritancy caused by ethanol can be substantially prevented.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the results of fluorescence microscopic observation in Comparative example 2.
FIG. 2 shows the results of fluorescence microscopic observation in Comparative example 3.
FIG. 3 shows the results of fluorescence microscopic observation in Comparative example 4.
FIG. 4 shows the results of fluorescence microscopic observation in Test example 2.
FIG. 5 shows the results of fluorescence microscopic observation in Comparative example 5.
FIG. 6 shows the results of fluorescence microscopic observation in Comparative example 3.
FIG. 7 shows the results of fluorescence microscopic observation in Test example 5.
FIG. 8 shows the results of fluorescence microscopic observation in Test example 5.

BEST MODE FOR CARRYING OUT THE INVENTION

Hereafter, embodiments of the present invention will be specifically described.

The composition for hair of the present invention is characterized in that it comprises protein nanoparticles containing an active ingredient for hair.

The type of active ingredient for hair used in the present invention is not particularly limited. However, the active ingredient can be selected from among cosmetic ingredients, quasi-drug ingredients, pharmaceutical ingredients, and the like. According to the present invention, specific examples of an active ingredient for hair contained in protein nanoparticles that can be selected may include moisturizing agents, ultraviolet absorbing agents, free-radical-removing agents, antioxidants, anti-inflammatory agents, blood circulation promoters, hair growth agents, hair nutritional supplements, anti-aging agents, collagen synthesis promoters, vitamins, minerals, and amino acids.

Examples of moisturizing agents include agar, diglycerin, deoxycholic acid, butylene glycol, polyethylene glycol, propylene glycol, hexylene glycol, Coix lachma-jobi extract, vaseline, urea, hyaluronic acid, ceramide, Lidipure, isoflavone, amino acid, collagen, mucopolysaccharide, lucidone, lactotetin, sorbitol, chitin/chitosan, malic acid, glucuronic acid, placenta extract, seaweed extract, moutan cortex extract, sweet tea extract, hypericin extract, colesus extract, Evonymus japonicus extract, salflower extract, Rosa rugosa flower extract, Polyergus sceleratus extract, hawthorn extract, rosemary extract, duke extract, chamomile extract, Lamium album extract, Litchi Chinesis extract, Achillea millefolium extract, aloé extract, marronner extract, Thujaospis dolabrata extract, Fucus extract, Osmian extract, oat bran extract, tuberosa polysaccharide, Cordyceps sinensis (plant worm) extract, barley extract, orange extract, Rehmannia glutinosa extract, anchoxyloxy extract, and Coix lachma-jobi extract. In addition, in the cases of casein nanoparticles, casein itself has moisture retention capacity.

Examples of ultraviolet protecting agents include homomethyl silicate, 4-methoxyxycinnamic acid-2-ethylhexyl, 2-hydroxy-4-methoxy benzophenone, 2-hydroxy-4-methoxy benzophenone sulfonic acid, 2-hydroxy-4-methoxy benzophenone sodium sulfonate, 4-t-butyl-4’-methoxydibenzoylmethane, titanium oxide, and zinc oxide.

Examples of free-radical-removing agents include superoxide dismutase (SOD), mannitol, carotenoids such as beta carotene, astaxanthin, rutin and derivatives thereof, bilirubin, cholesterol, tryptophan, histidine, quercetin, quercitin, catechin, catechin derivatives, gallic acid, gallic acid derivatives, Scutellariae radix extract, ginkgo extract, Saxifragna stolonifera (strawberry geranium) extract, melissa extract, Geranium thunbergii extract, moutan cortex extract, parsley extract, tormentilla extract, Monordica grosvenori extract, seaweed extract, Yashajitsu (Alnus firma Sieb. et Zucc.) extract, and Lycii Cortex extract.

Examples of antioxidants include carotenes, retinoic acid, retinol, vitamin C and derivatives thereof, kinetin, astaxanthin, tretinoin, vitamin E and derivatives thereof, sesamin, alpha-lipoic acid, coenzyme Q10, flavonoids, erythorbic acid, gallic acid propyl, BHT (di-n-butylhydroxytoluene), BHA (butylhydroxyanisole), Engelhardia chrysophleps Hance extract, soybean extract, black tea extract, green tea extract, and Rosae multiflorae fructus extract.

Examples of anti-inflammatory agents include: compounds and salts and derivatives thereof selected from the group consisting of azulene, guaiazulene, diphenhydramine hydrochloride, hydrocortisone acetate, prednisolone, glyceryl bice, glyceryl bice, mafenamic acid, phenylbutazone, indomethacin, ibuprofen, and ketoprofen; and plant extracts selected from the group consisting of Scutellariae radix extract, Artemisia capillaris extract, balloonflower (Platycodon grandiflorus) extract, Armeriaaceae semen extract, gardenia extract, Sasa veitchii extract, gentiana extract.
extract, comfrey extract, white birch extract, mallow extract, 
Persicae semen extract, peach leaf extract, and Erinobryae 
follium extract.

Examples of blood circulation promoters that can be 
selected include nicotinic acid, Svertia japonica extract, 
γ-oxazole, alkoxybenzyldine N-oxide, carpronium 
chloride, and acetylcysteine or derivatives thereof.

Examples of hair growth agents include glycyrrethic 
acid or derivatives thereof, glycyrrethic acid or derivatives 
thereof, hinokitiol, minoxidil and analogs thereof, adenosine, 
vitamin E and derivatives thereof, vitamin C derivatives, 
6-benzyl aminopurine, nicotinic acid benzyl, nicotinic acid 
tocopherol, nicotinic acid β-2-butoxyster, isopropyl meth-
lyphenol, pentadecanoic acid and derivatives thereof, cepha-
latin, finasteride, t-hexamone, and pantethenyl ethyl ether. 
Among them, hinokitiol and minoxidil or analogs thereof 
are most preferable.

Also, known ingredients can be used as anti-aging 
agents, collagen synthesis promoters, vitamins, minerals, 
and amino acids.

The above active ingredients for hair may be used 
alone or in combinations of two or more.

According to the present invention, it was found 
that, with the use of interaction between a fat-soluble 
active ingredient for hair and a casein hydrophobic domain, it is 
possible for casein nanoparticles to contain the active ingredient 
for hair. Further, it was found that such particles remain 
stable in an aqueous solution. The Clog P of a fat-soluble 
substance is preferably more than 0 and more preferably 
not less than 1.

Further, it was found that a particle mixture of 
protein and ionic polysaccharide or another ionic protein can 
contain an ionic active ingredient for hair.

The composition for hair of the present invention 
comprises preferably 0.01% to 50% by weight and most 
preferably 0.1% to 10% by weight protein nanoparticles.

The composition for hair of the present invention 
contains an active ingredient for hair in a weight that 
is preferably 0.1% to 100% and more preferably 0.1% to 50% 
of the protein weight.

According to the present invention, an active ingre-
dient for hair may be added during or after protein nanopar-
ticle formation.

Further, the composition for hair of the present 
invention may comprise, as an additive, an active ingredient 
for hair. Specific examples of active ingredients for hair that 
serve as additives include, but are not limited to, pantothenic 
acid, panthenol, licorice extract, Lepisorus thunbergianus 
extract, Sophora Radix (sophora root) extract, Svertia 
janonica extract, capsicum extract, Ampelopsis cianonis 
var. grossedentata extract, carrot extract, Taxacum mong-
golicum Hand.-Mazz. extract, tree peony extract, and man-
darin orange extract.

The average particle size of protein nanoparticles 
used in the present invention is generally 1 to 1000 nm, 
preferably 10 to 1000 nm, more preferably 10 to 200 nm, 
further preferably 10 to 100 nm, and particularly preferably 
20 to 50 nm.

The type of protein used in the present invention 
is not particularly limited. However, a protein having a lysine 
residue and a glutamic residue is preferable. In addition, 
such protein having a molecular weight of approximately 
10,000 to 1,000,000 is preferably used. The origin of the 
protein is not particularly limited. However, a human-derived 
protein is preferably used. Specific examples of a protein that 
can be used include, but are not limited to, the following 
compounds according to the present invention: at least one 
selected from the group consisting of collagen, gelatin, acid-
treated gelatin, albumin, ovalbumin, casein, transferrin, 
globulin, fibron, fibrin, laminin, fibronectin, and vitronectin.

In addition, the origin of the protein is not particularly limited. Thus, any bovine, swine, or fish protein, as well as recombi-
nant protein of any thereof, can be used. Examples of recombi-
nant gelatin that can be used include, but are not limited to, 
gelatins described in EP1014176 A2 and U.S. Pat. No. 6,992, 
172. Among them, casein, acid-treated gelatin, collagen, 
and albumin is preferable. Further, casein or acid-treated gelatin 
is most preferable.

Upon the use of casein according to the present 
invention, the origin of the casein is not particularly limited. 
Casein may be milk-derived or bean-derived. Any of α-casein, β-casein, γ-casein, and κ-casein, as well as a mixture 
of any thereof, can be used. Also, a recombinant thereof 
can be used. Preferably, casein sodium can be used. Caseins 
may be used alone or in combinations of two or more.

Proteins used in the present invention may be used 
alone or in combinations of two or more.

According to the present invention, it is possible to 
carry out a crosslinking treatment for a protein during and/or 
after nanoparticle formation. For the crosslinking treatment, 
an enzyme can be used. Any enzyme may be used without 
particular limitation as long as it has been known to have the 
effect of causing protein crosslinking. Among such enzymes, 
transglutaminase is preferable.

Transglutaminase may be derived from a mammal 
or a microorganism. A recombinant transglutaminase can be 
used. Specific examples thereof include the Activa series by 
Ajinomoto Co., Inc., commercially available mammalian-
derived transglutaminase serving as a reagent, such as guinea 
pig liver-derived transglutaminase, goat-derived trans-
glutaminase, rabbit-derived transglutaminase, or human-
derived recombinant transglutaminase produced by, for 
example, Oriental Yeast Co., Ltd., Upstate USA Inc., and 
Biodesign International.

The amount of an enzyme used in a crosslinking 
treatment according the present invention can be adequately 
determined depending upon protein type. In general, 
an enzyme can be added in a weight that is 0.1% to 100% 
and preferably approximately 1% to 50% of the protein weight.

The duration for an enzymatic crosslinking reaction 
can be adequately determined depending upon protein type 
and nanoparticle size. However, in general, the reaction can 
be carried out for 1 to 72 and preferably 2 to 24 hours.

The temperature for an enzymatic crosslinking reac-
tion can be adequately determined depending upon protein 
type and nanoparticle size. In general, the reaction can 
be carried out at 0°C to 80°C and preferably at 25°C to 60°C.

Enzymes used in the present invention may be used 
alone or in combinations of two or more.

Nanoparticles of the present invention can be pre-
pared in accordance with Patent Document: JP Patent Publi-
cation (Kokai) No. 6-79168 A (1994); or C. Coester, Journal 
Microcapsulation, 2000, vol. 17, pp. 187-193, provided that 
an enzyme is preferably used instead of glutaraldehyde for a 
crosslinking method.

In addition, according to the present invention, the 
enzymatic crosslinking treatment is preferably carried out in 
an organic solvent. The organic solvent used herein is 
preferably an aqueous organic solvent such as ethanol, isopro-
panol, acetone, or THF.

Further, according to the present invention, it is 
preferable to remove an organic solvent by distillation 
subsequent to a crosslinking treatment, followed by water 
dispersion. It is
also possible to add water prior to or subsequent to removal of an organic solvent by distillation.

[0075] It is also possible to add at least one component selected from the group consisting of lipids (e.g., phospholipid), anionic polysaccharides, cationic polysaccharides, anionic proteins, cationic proteins, and cyclodextrin to the composition for hair of the present invention. The amounts of lipid (e.g. phospholipid), anionic polysaccharide, cationic polysaccharide, anionic protein, cationic protein, and cyclodextrin to be added are not particularly limited. However, they can be added usually in a weight that is 0.1% to 100% of the protein weight. In the case of the composition for hair of the present invention, it is possible to adjust the release rate by changing the ratio of the above components to the protein.

[0076] Specific examples of phospholipids that can be used in the present invention include, but are not limited to, the following compounds according to the present invention: phosphatidylycholine (lecithin), phosphatidylethanolamine, phosphatidylerine, phosphatidylcholinol, phosphatidylglycerol, diphosphatidyglycerol, and sphingomyelin.

[0077] Anionic polysaccharides that can be used in the present invention are polysaccharides having an acidic polar group such as a carboxyl group, a sulfonic group, or a phosphoric group. Specific examples thereof include, but are not limited to, the following compounds according to the present invention: chondroitin sulfate, dextran sulfate, carboxymethyl cellulose, carboxymethyl dextran, alginate, peetin, carrageenan, fucoidan, agaropeptin, porphyran, karaya gum, gellan gum, xanthan gum, and hyaluronic acids.

[0078] Cationic polysaccharides that can be used in the present invention are polysaccharides having a basic polar group such as an amino group. Examples thereof include, but are not limited to, the following compounds according to the present invention: polysaccharides such as chitin or chitosan, which comprise, as a monosaccharide unit, glucosamine or galactosamine.

[0079] Anionic proteins that can be used in the present invention are proteins and lipoproteins having a more basic isoelectric point than the physiological pH. Specific examples thereof include, but are not limited to, the following compounds according to the present invention: polyglutamic acid, polypeptides, lysozyme, lysozyme C, ribonuclease, trypsinogen, chymotrypsinogen, and α-chymotrypsin.

[0080] Cationic proteins that can be used in the present invention are proteins and lipoproteins having a more acidic isoelectric point than the physiological pH. Specific examples of such cationic protein include, but are not limited to, the following compounds according to the present invention: polylysine, polyarginine, histone, protamine, and ovalbumin.

[0081] According to the present invention, it is possible to use casein nanoparticles prepared by the following steps [(a) to (c)]:

[0082] (a) mixing casein with a basic aqueous medium at pH of 8 to less than 11;
[0083] (b) adding at least one active ingredient for hair to the solution obtained in step;
[0084] (a); and
[0085] (c) injecting the solution obtained in step (b) into an acidic aqueous medium at pH of 3.5 to 7.5.

[0086] Further, according to the present invention, it is possible to use casein nanoparticles prepared by the following steps [(a) to (c)]:

[0087] (a) mixing casein with a basic aqueous medium at pH of 8 to less than 11;
[0088] (b) adding at least one active ingredient for hair to the solution obtained in step;
[0089] (a); and
[0090] (c) lowering the pH of the solution obtained in step (b) to pH value which is different from the isoelectric point by 1 or more units, while stirring the solution.

[0091] According to the present invention, it is possible to prepare casein nanoparticles of desired sizes. Also, with the use of interaction between a hydrophobic active ingredient for hair and a casein hydrophobic domain, it is possible for casein nanoparticles to contain the active ingredient for hair. In addition, it was found that such particles remain stable in an aqueous solution.

[0092] Further, it was found that a particle mixture of casein and ionic polysaccharide or another ionic protein can contain an ionic active ingredient for hair.

[0093] The method for preparing casein nanoparticles of the present invention involves a method wherein casein is mixed with a basic aqueous medium solution and the solution is injected into an acidic aqueous medium, and a method wherein casein is mixed with a basic aqueous medium and the pH of the medium is lowered during stirring, for example.

[0094] The method wherein casein is mixed with a basic aqueous medium solution and the solution is injected into an acidic aqueous medium is preferably carried out using a syringe for convenience. However, there is no particular limitation as long as the injection rate, solubility, temperature, and stirring conditions are satisfied. Injection can be carried out usually at an injection rate of 1 mL/min to 100 mL/min. The temperature of the basic aqueous medium can be adequately determined. In general, the temperature is 0°C to 80°C and preferably 25°C to 70°C. The temperature of an aqueous medium can be adequately determined. In general, the temperature can be 0°C to 80°C and preferably 25°C to 60°C. The stirring rate can be adequately determined. However, in general, the stirring rate can be 100 rpm to 3000 rpm and preferably 200 rpm to 2000 rpm.

[0095] In the method wherein casein is mixed with a basic aqueous medium and the pH of the medium is lowered during stirring, it is preferable to add acid dropwise for convenience. However, there is no particular limitation as long as solubility, temperature, and stirring conditions are satisfied. The temperature of a basic aqueous medium can be adequately determined. However, in general, the temperature can be 0°C to 80°C and preferably 25°C to 70°C. The stirring rate can be adequately determined. However, in general, the stirring rate can be 100 rpm to 3000 rpm and preferably 200 rpm to 2000 rpm.

[0096] The aqueous medium that can be used for the present invention is an aqueous solution or a buffer comprising an organic acid or base or an inorganic acid or base.

[0097] Specific examples thereof include, but are not limited to, aqueous solutions comprising: organic acids such as citric acid, ascorbic acid, gluconic acid, carboxylic acid, tartaric acid, succinic acid, acetic acid, phthalic acid, and fumaric acid; and phospholipids; and 2,4-(2-hydroxyethyl)-1-piperazineyl-2-hydroxyethylsulfonic acid; and organic bases such as triis (hydroxyethyl), aminomethane, and ammonia; and inorganic acids such as hydrochloric acid, perchloric acid, and carboxylic acid; and ammonium or organic bases such as sodium phosphate, potassium phosphate, calcium hydroxide, sodium hydroxide, potassium hydroxide, and magnesium hydroxide.

[0098] The concentration of an aqueous medium used in the present invention is preferably approximately 10 mM to 1 M, and more preferably approximately 20 mM to 200 mM.

[0099] The pH of a basic aqueous medium used in the present invention is preferably 8 or more, preferably 8 to 12, and further preferably 10 to 12. When the pH is exces-
sively high, there is concern regarding hydrolysis or risks in handling. Thus, the pH is preferably in the above range.

[0100] According to the present invention, the temperature at which casein is mixed with a basic aqueous medium at a pH of 8 or more is preferably 0°C to 10°C, more preferably 10°C to 30°C, and further preferably 20°C to 70°C.

[0101] The pH of an acidic aqueous medium used in the present invention is preferably 3.5 to 7.5 and more preferably 5 to 6. When the pH does not fall in the above range, the particle size tends to become large.

[0102] The composition for hair of the present invention may further comprise an additive. Examples of an additive that can be used include, but are not limited to, at least one selected from the group consisting of softening agents, transdermal absorption enhancers, soothing agents, preservatives, antioxidants, coloring agents, thickeners, aroma chemicals, and pH adjusters.

[0103] Specific examples of softening agents that can be used in the present invention include, but are not limited to, the following compounds according to the present invention: glycerin, mineral oil, and emollient ingredients (e.g., isopropyl isostearate, polyglyceryl isostearate, isododecyl isononanoate, octyl isononanoate, oleic acid, glyceryl oleate, cocoa butter, cholesterol, mixed fatty acid triglyceride, dioctyl succinate, sucrose tetraesterate triacetate, cyclomethicone, sucrose distearate, palmitateoctyl, octyl hydroxystearate, arachidyl behenate, sucrose polybehenate, polymethyleisocyclohexane, myristyl alcohol, cetyl myristate, myristyl myristate, and hexyl laurate).

[0104] Specific examples of transdermal absorption enhancers that can be used in the present invention include, but are not limited to, the following compounds according to the present invention: ethanol, isopropyl myristate, citric acid, squalane, oleic acid, menthol, N-methyl-2-pyrrolidone, diethyl adipate, disopropyl adipate, diethyl sebacate, diso-
propyl sebacate, isopropyl palmitate, oleic acid isopropyl, oleic acid cetyldecyl, isostearyl alcohol, 2-octidodecanol, urea, vegetable oil, and animal oil.

[0105] Specific examples of soothing agents that can be used in the present invention include, but are not limited to, the following compounds according to the present invention: benzyl alcohol, procaine hydrochloride, xyloicaine hydrochloride, and chlorbutanol.

[0106] Specific examples of preservatives that can be used in the present invention include, but are not limited to, the following compounds according to the present invention: benzoic acid, sodium benzoate, parabens, ethylparaben, methylparaben, propylparaben, butylparaben, potassium sorbate, sodium sorbate, sorbic acid, sodium dehydroacetate, hydrogen peroxide, formaldehyde, ethyl formate, sodium hydrochloride, propionic acid, sodium propionate, calcium propionate, pectin degradation products, polylene, phenol, isopropylmethylphenol, orthophenylphenol, phenoxyethanol, resorcin, dibutylhydroxytoluene (BHT), thymol, thiram, tea tree oil, hinokitiol, glycerin, dipropylene glycol, 1,3-butylene glycol, 1,4-butylene glycol, 1,2pentanediol, and 2-methyl-2,4pentanediol.

[0107] Specific examples of antioxidants that can be used in the present invention include, but are not limited to, the following compounds according to the present invention: vitamin A, retinoic acid, retinol, retinol acetate, retinol palmi-
tate, retinyl acetate, retinyl palmitate, tocopheryl retinocate, vitamin C and derivatives thereof, kinetin, β-carotene, astax-
athanin, lutein, lycopene, tretinoin, vitamin E, α-lipoic acid, coenzyme Q10, polyphenol, SOD, and phytic acid.

[0108] Specific examples of coloring agents that can be used in the present invention include, but are not limited to, the following compounds according to the present invention: krill pigment, orange dye, cacao dye, kaoline, carmines, ultramarine blue, cochineal dye, chrome oxide, iron oxide, titanium dioxide, tar dye, and chlorophyll.

[0109] Specific examples of thickeners that can be used in the present invention include, but are not limited to, the following compounds according to the present invention: quince seed, carrageenan, gum arabic, karaya gum, xanthan gum, gellan gum, tamarind gum, locust bean gum, gum tragacanth, pectin, starch, cyclodextrin, methylcellulose, ethylcellulose, carboxymethylcellulose sodium, sodium alginate, polyvinyl alcohol, polyvinyl pyrrolidone, carboxyvinyl polymer, and sodium polyacrylate.

[0110] Specific examples of aroma chemicals that can be used in the present invention include, but are not limited to, the following compounds according to the present invention: musk, acacia oil, anise oil, ylang ylang oil, cinnamon oil, jasmine oil, sweet orange oil, spearmint oil, geranium oil, thyme oil, neroli oil, mentha oil, hinoki (Japanese cypress) oil, fennel oil, peppermint oil, bergamot oil, lime oil, lavender oil, lemon oil, lemongrass oil, rose oil, rosewood oil, anisaldehyde, geraniol, citral, cedrione, muscone, limonene, and vanillin.

[0111] Specific examples of pH adjusters that can be used in the present invention include, but are not limited to, the following compounds according to the present invention: sodium citrate, sodium acetate, sodium hydroxide, potassium hydroxide, phosphoric acid, and succinic acid.

[0112] Examples of the dosage form of the composition for hair of the present invention include, but are not limited to, liquid formulations for external use, emulsions, emulsions, bathing agents, bath additives, disinfectants, ointments, gels, creams, pastes, adhesive skin patches, plasters, wound surface-covering agents, wound surface-covering gauzes, hemostatics, adhesives, adhesive tape, adhesive tape for transdermal absorption, wound protective agents, aerosols, lotions, tonics, liniments, emulsions, suspensions, saturants, textures, powders, foaming agents, skin lotions, massage creams, nourishing creams, face packs, sheet-type drugs for external use, cosmetics for makeup, skin coloring agents for external use, cosmetic skin adhesives, shampoos, rinses, hair conditioners, hair packs, hair liquids, hair tonics, hair sprays, permanent wave compositions, hair dyes, body soap, soap, bath agents, sun care products (e.g., sunscreens, sun tanning oils, and after-sun lotions), and fragrances.

[0113] The composition for hair of the present invention can be administered without particular limitation. For instance, it can be administered by directly applying it to the scalp.

[0114] The dose of the composition for hair of the present invention can be adequately determined depending upon type and amount of active ingredient for hair and upon user weight and condition, for example. The dose for single administration is generally approximately 1 µg to 50 mg/cm² and preferably 2.5 µg to 10 mg/cm².

[0115] The ethanol content in the composition for hair of the present invention is preferably 20% by weight or less, more preferably 10% by weight or less, and most preferably substantially 0% by weight.

[0116] The present invention is hereafter described in greater detail with reference to the following examples, although the technical scope of the present invention is not limited thereto.

EXCEPTIONS

Example 1

[0117] Milk-derived casein (100 mg, Wako Pure Chemical Industries, Ltd.) was mixed with 50 mM phosphate buffer (pH

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Coumarin 6 (0.015 mg: Wako Pure Chemical Industries, Ltd.) was dissolved in ethanol (0.1 mL). The two different solutions were mixed together. Hydrochloric acid was added thereto so that the pH was adjusted to 7.5. Thus, casein nanoparticles were obtained.

The average particle size of the above particles was measured with a “Microtrac” light scattering photometer (NIKKISO Co., Ltd.) and found to be 29 nm.

Example 2

Milk-derived casein Na (10 mg; Wako Pure Chemical Industries, Ltd.) was dissolved with 50 mM phosphate buffer (pH 9, 1 mL). Glycyrrhizic acid (1.7 mg; Wako Pure Chemical Industries, Ltd.) was dissolved in ethanol (0.25 mL). The glycyrrhizic acid solution was added dropwise to the casein solution during stirring. The resulting liquid mixture (1 mL) was injected into 200 mM phosphate buffer water (pH 5, 10 mL) with the use of a microsyringe at an external temperature of 40°C during stirring at 800 rpm. Thus, a water dispersion of casein nanoparticles containing glycyrrhizic acid was obtained. The average particle size of the above particles was measured with a “Microtrac” light scattering photometer (NIKKISO Co., Ltd.) and found to be 83 nm.

Example 3

Milk-derived casein Na (10 mg; Wako Pure Chemical Industries, Ltd.) was dissolved with 50 mM phosphate buffer (pH 9, 1 mL). Hinokitiol (1.7 mg; Wako Pure Chemical Industries, Ltd.) was dissolved in ethanol (0.25 mL). The hinokitiol solution was added dropwise to the casein solution during stirring. The resulting liquid mixture (1 mL) was injected into 200 mM phosphate buffer water (pH 5, 10 mL) with the use of a microsyringe at an external temperature of 40°C during stirring at 800 rpm. Thus, a water dispersion of casein nanoparticles containing hinokitiol was obtained. The average particle size of the above particles was measured with a “Microtrac” light scattering photometer (NIKKISO Co., Ltd.) and found to be 57 nm.

Example 4

Milk-derived casein Na (10 mg; Wako Pure Chemical Industries, Ltd.) was dissolved with 50 mM phosphate buffer (pH 9, 1 mL). Tocopherol acetate (1.7 mg) was dissolved in ethanol (0.25 mL). The tocopherol acetate solution was added dropwise to the casein solution during stirring. The resulting liquid mixture (1 mL: casein solution) was injected into 200 mM phosphate buffer water (10 mL) with the use of a microsyringe at an external temperature of 40°C during stirring at 800 rpm. Thus, a water dispersion of casein nanoparticles containing tocopherol acetate was obtained. The average particle size of the above particles was measured with a “Microtrac” light scattering photometer (NIKKISO Co., Ltd.) and found to be 124 nm.

Example 5

Milk-derived casein Na (10 mg; Wako Pure Chemical Industries, Ltd.) was dissolved with 50 mM phosphate buffer (pH 9, 1 mL). The casein solution (1 mL) was injected into 200 mM phosphate buffer water (pH 5, 10 mL) in which minoxidil (1.7 mg) had been dissolved with the use of a microsyringe at an external temperature of 40°C during stirring at 800 rpm. Thus, a water dispersion of casein nanoparticles containing minoxidil was obtained. The average particle size of the above particles was measured with a “Microtrac” light scattering photometer (NIKKISO Co., Ltd.) and found to be 55 nm.

Example 6

Acid-treated gelatin (10 mg) and TG-S (5 mg; Ajinomoto Co., Inc.) were dissolved in water (1 mL). The gelatin solution (1 mL) was injected into ethanol (10 mL) in which glycyrrhizic acid (1.7 mg) had been dissolved with the use of a microsyringe at an external temperature of 40°C during stirring at 800 rpm. Thus, gelatin nanoparticles were obtained. The gelatin nanoparticles were allowed to stand at an external temperature of 55°C for 5 hours for enzymatic crosslinking. The average particle size of the above particles was measured with a “Microtrac” light scattering photometer (NIKKISO Co., Ltd.) and found to be 80 nm.

Example 7

Water (5 mL) was added to the obtained gelatin nanoparticle dispersion and ethanol was removed therefrom by means of a rotary evaporator. Thus, a water dispersion of gelatin nanoparticles containing glycyrrhizic acid was obtained. The average particle size of the above particles was measured with a “Microtrac” light scattering photometer (NIKKISO Co., Ltd.) and found to be 201 nm.

Example 8

Milk-derived casein (100 mg; Wako Pure Chemical Industries, Ltd.) was dissolved with 50 mM phosphate buffer (pH 10, 10 mL). Hyaluronic acid (1 mg; Wako Pure Chemical Industries, Ltd.) was dissolved in the solution. Hydrochloric acid was added thereto so that the pH was adjusted to 7. Thus, casein nanoparticles were obtained.

The average particle size of the above particles was measured with a “Microtrac” light scattering photometer (NIKKISO Co., Ltd.) and found to be 23 nm.

Example 9

Milk-derived casein (100 mg; Wako Pure Chemical Industries, Ltd.) was dissolved with 50 mM phosphate buffer (pH 10, 10 mL). Palmitoylascorbic acid (1 mg; Wako Pure Chemical Industries, Ltd.) was dissolved in ethanol (0.2 mL). The two different solutions were mixed together. Hydrochloric acid was added thereto so that the pH was adjusted to 7. Thus, casein nanoparticles were obtained.

The average particle size of the above particles was measured with a “Microtrac” light scattering photometer (NIKKISO Co., Ltd.) and found to be 30 nm.

Example 10

Acid-treated gelatin (10 mg) and TG-S (5 mg; Ajinomoto Co., Inc.) were dissolved in water (1 mL). The gelatin solution (1 mL) was injected into ethanol (10 mL) in which tocopherol (1.7 mg) had been dissolved with the use of a microsyringe at an external temperature of 40°C during stirring at 800 rpm. Thus, gelatin nanoparticles were obtained. The gelatin nanoparticles were allowed to stand at an external temperature of 55°C for 5 hours for enzymatic crosslinking.

The average particle size of the above particles was measured with a “Microtrac” light scattering photometer (NIKKISO Co., Ltd.) and found to be 95 nm.

Milk-derived casein (100 mg; Wako Pure Chemical Industries, Ltd.) was dissolved with 50 mM phosphate buffer (pH 10, 10 mL). Pantothenyl ethyl ether (400 mg; Wako Pure
Chemical Industries, Ltd.) was dissolved in ethanol (0.8 mL). The two different solutions were mixed together. Hydrochloric acid was added thereto so that the pH was adjusted to 6. Thus, casein nanoparticles were obtained.

[0132] The average particle size of the above particles was measured with a "Nano-ZS" light scattering photometer (Malvern Instruments Ltd.) and found to be 24 nm.

Test Example 1

[0133] The dispersions of nanoparticles containing active substances for hair described in Examples 2 to 6 were preserved at room temperature for 1 month. Thereafter, average particle size measurement was carried out using a Microtrac (NIKKISO Co., Ltd.).

[0134] As Comparative example 1, a "NanoImpact" synthetic polymer (PLGA) nanoparticle dispersion (Hosokawa Micron Corporation) was used.

[0135] Table 1 shows measurement results obtained in Test example 1.

| TABLE 1 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Comparative     | Example 1       | Example 2       | Example 3       | Example 4       |
| When prepared  | nm              | nm              | nm              | nm              | nm              |
| 1 month later  | N.D.            | 90 nm           | 63 nm           | 141 nm          | 62 nm           |

N.D.: Not detected

Test Example 2

Hairless Rat Excised Skin Test

[0136] A 2-cm square piece of nonwoven cloth absorbing 400 μL of the casein nanoparticle dispersion prepared in Example 1 was applied to hairless rat excised skin and the skin was allowed to stand for 30 minutes. Then, the skin was embedded within an OCT compound (Sakura Finetek Co., Ltd.) and frozen with liquid nitrogen. A frozen section was prepared from the skin with the use of a cryostat (Carl Zeiss). Then, the section was immobilized and enclosed on a prepared slide with the use of a DAPI-containing mounting agent, followed by fluorescence microscopic observation.

Comparative Example 2

No Application

Comparative Example 3

[0137] With the use of the following dispersion, fluorescence microscopic observation was conducted as with Test example 2.

[0138] Milk-derived casein (100 mg; Wako Pure Chemical Industries, Ltd.) was mixed with distilled water (10 mL). Coumarin 6 (0.015 mg; Wako Pure Chemical Industries, Ltd.) was dissolved in ethanol (0.1 mL). A dispersion was obtained by mixing the two different solutions.

Comparative Example 4

[0139] With the use of the following solution, fluorescence microscopic observation was conducted as with Test example 2.

[0140] A solution was obtained by dissolving coumarin 6 at a concentration of 0.15 mg/mL in a 50% ethanol aqueous solution.

Test Example 3

SD Rat in Vivo Test

[0141] A SD rat was subjected to anesthetic injection. Thereafter, a 2-cm square piece of nonwoven cloth absorbing 400 μL of the casein nanoparticle dispersion prepared in Example 1 was applied to the abdominal skin of the rat and the skin was allowed to stand for 60 minutes. The skin was embedded within an OCT compound (Sakura Finetek Co., Ltd.) and frozen with liquid nitrogen. A frozen section was prepared from the skin with the use of a cryostat (Carl Zeiss). Then, the section was immobilized and enclosed on a prepared slide with the use of a DAPI-containing mounting agent, followed by fluorescence microscopic observation.

Comparative Example 5

[0142] With the use of the following solution, fluorescence microscopic observation was conducted as with Test example 3.

[0143] A solution was obtained by dissolving coumarin 6 at a concentration of 0.15 mg/mL in a 50% ethanol aqueous solution.

[0144] FIGS. 1a to 6a show fluorescence photomicrographs of the hairless rat excised skins and the SD rat skin sections in Comparative examples 2, 3, 4, and 5 and Test examples 2 and 3. FIGS. 1b to 6b show DAPI-stained tissue images corresponding to the visual fields in FIGS. 1a to 6a.

Test Example 4

Dorsal hair of C57 mice at the trichogenous or dormant phase were cut with a hair clipper. On the next day, the mice were shaved with a shaver. The water dispersions of protein nanoparticles containing hair growth agents prepared in Examples 2 to 5 were separately applied to all shaved areas once daily. The degree of ability to cause phase transition to the growth phase in mouse dorsal hair follicles was examined. As a result, hair growth was promoted and activity of causing hair cycle transition from the dormant phase to the growth phase was observed.

Test Example 5

Coumarin 6 at a concentration of 0.15 mg/mL was dissolved in the dispersion prepared in Example 1 and in a 50% ethanol aqueous solution. The obtained solutions at 25°C were separately applied to goat hair. After 30 minutes of reaction time, goat hair was washed and dried. The color tone of the obtained dyed hair was observed. In the case of Example 1, good permeation was confirmed. FIG. 7a shows a fluorescence photomicrograph of a goat hair surface (Example 1). FIG. 7b shows fluorescence photomicrograph of a goat hair cross section. FIG. 8a shows a fluorescence photomicrograph of a goat hair surface (ethanol solution). FIG. 8b shows fluorescence photomicrograph of a goat hair cross section.

Test Example 6

Sensory Evaluation

[0147] A human hair bundle 15 cm in length, 1 cm in width, and 1 g in weight was immersed in the composition (5 mL) obtained in Example 10 for approximately 30 seconds and sufficiently dried. The hair bundle was designated as a test
hair bundle. An untreated hair bundle was designated as a reference hair bundle. The hair bundles were subjected to five-grade sensory evaluation in terms of smoothness, elasticity, volume/ strength, and uniformity in accordance with the criteria listed in Table 2. Evaluation was carried out by 5 persons. The mean values are listed in Table 3.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Evaluation criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Smoothness)</td>
<td>(Elasticity)</td>
</tr>
<tr>
<td>4: Sufficent smoothness</td>
<td>4: Test hair: Superior in elasticity</td>
</tr>
<tr>
<td>3: Normal smoothness</td>
<td>3: Test hair: Slightly superior in elasticity</td>
</tr>
<tr>
<td>2: No obvious difference</td>
<td>2: No obvious difference</td>
</tr>
<tr>
<td>1: Poor smoothness</td>
<td>1: Reference hair: Slightly superior in elasticity</td>
</tr>
<tr>
<td>0: No smoothness</td>
<td>0: Reference hair: Superior in elasticity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(Volume/Strength)</th>
<th>(Uniformity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4: Test hair: Superior in volume and strength</td>
<td>4: Test hair: Superior in uniformity</td>
</tr>
<tr>
<td>3: Test hair: Slightly superior in volume and strength</td>
<td>3: Test hair: Slightly superior in uniformity</td>
</tr>
<tr>
<td>2: No obvious difference</td>
<td>2: No obvious difference</td>
</tr>
<tr>
<td>1: Reference hair: Slightly superior in volume and strength</td>
<td>1: Reference hair: Slightly superior in uniformity</td>
</tr>
<tr>
<td>0: Reference hair: Superior in volume and strength</td>
<td>0: Reference hair: Superior in uniformity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Sensory evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoothness</td>
<td>Elasticity</td>
</tr>
<tr>
<td>Test hair</td>
<td>3.8</td>
</tr>
<tr>
<td>Reference hair</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1. A composition for hair which comprises protein nanoparticles containing an active ingredient for hair.

2. The composition for hair according to claim 1 wherein the average particle size of protein nanoparticles is 10 to 1000 nm.

3. The composition for hair according to claim 1 wherein the protein nanoparticles contain an active ingredient for hair in a weight that is 0.1% to 100% of the protein weight.

4. The composition for hair according to claim 1 wherein the active ingredient for hair is at least one selected from the group consisting of cosmetic ingredients, quasi-drug ingredients, and pharmaceutical ingredients.

5. The composition for hair according to claim 1 wherein the active ingredient for hair is an ionic substance or a small-soluble substance.

6. The composition for hair according to claim 1 wherein the active ingredient for hair is a hair growth agent.

7. The composition for hair according to claim 1 wherein the ethanol content is 20% by weight or less.

8. The composition for hair according to claim 1 wherein the protein is at least one selected from the group consisting of collagen, gelatin, acid-treated gelatin, albumin, ovalbumin, casein, transferrin, globulin, fibrin, fibrin, laminin, fibronectin, and vitronectin.

9. The composition for hair according to claim 1 wherein the protein is subjected to crosslinking treatment during and/or after nanoparticle formation.

10. The composition for hair according to claim 9 wherein crosslinking treatment is carried out by an enzyme.

11. The composition for hair according to claim 1 which comprises casein nanoparticles prepared by the following steps (a) to (c):

   (a) mixing casein with a basic aqueous medium at pH of from 8 to less than 11;

   (b) adding at least one active ingredient for hair to the solution obtained in step (a); and

   (c) injecting the solution obtained in step (b) into an acidic aqueous medium at pH of 3.5 to 7.5:

12. The composition for hair according to claim 1 which comprises casein nanoparticles prepared by the following steps (a) to (c):

   (a) mixing casein with a basic aqueous medium at pH of from 8 to less than 11;

   (b) adding at least one active ingredient for hair to the solution obtained in step (a); and

   (c) lowering the pH of the solution obtained in step (b) to pH value which is different from the isoelectric point by 1 or more units, while stirring the solution.

* * * * *