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SEMI-SOLID PHARMACEUTICAL AGENT AND PROCESS TO PRODUCE THE SAME
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- (57) Claim

1. A semi-solid pharmaceutical composition in the form of an ointment, cachou or suppository which is administered to a subject through a percutaneous or permucosal route, said composition comprising a pharmaceutically-acceptable oligosaccharide, a proteinaceous bioactive substance as an effective ingredient for medical use, and a pharmaceutically-acceptable oil and fat base, wherein said proteinaceous bioactive substance is stabilised by said oligosaccharide and said oil and fat base, and said composition containing with respect to one part by weight of said oligosaccharide, 0.02 or lower part by weight of said proteinaceous bioactive substance and 2 or higher parts by weight of said oil and fat base.

6. A process to prepare a semi-solid pharmaceutical composition, which comprises mixing an oligosaccharide and a solution of a proteinaceous bioactive substance, and kneading the resultant solid with an oil and fat base.

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COMPLETE SPECIFICATION
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TO BE COMPLETED BY APPLICANT



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Invention Title: "SEMI-SOLID PHARMACEUTICAL AGENT AND PROCESS TO
PRODUCE THE SAME"

The following statement is a full description of this invention, including the best method of performing it known to me:-

**Semi-solid pharmaceutical agent and
process to produce the same**

Background of the Invention

Field of the Invention

The present invention relates to a semi-solid pharmaceutical agent and a process to produce the same, in particular, a stabilized semi-solid pharmaceutical agent with proteinaceous bioactive substances and a process to produce the same.

Description of the Prior Art

Semi-solid pharmaceutical agents containing chemicals as effective ingredient such as those in ointment or suppository form have been produced hitherto by simply kneading effective ingredients and bases. In case that effective ingredients are proteinaceous bioactive substances such as cytokines and hormones which are relatively labile, unlike chemicals, if one kneads them into semi-solid, then he or she will find that they have been already inactivated or they are still susceptible to inactivation and this renders such a semi-solid infeasible in actual uses.

Because of this, proteinaceous bioactive substances have been prepared into liquid forms where their activities were stably retained and then administered by injection through subcutaneous or intramuscular route in order for treatment of

susceptive diseases, for example, immunopathies, viral disease, abnormalities in hormone secretion and malignant tumors.

While proteinaceous bioactive substances were found latter to be very efficacious in treatment of trauma in the skin and mucous membranes as well as of such a susceptible disease, therefore other administration methods such as percutaneous and permucosal injections, which are usually deemed to be safer and less in pain, have been preferred. Thus development of semi-solid pharmaceutical agents using proteinaceous bioactive substances in stabilized form have been in great demand.

Summary of the Invention

The present invention relates to a semi-solid pharmaceutical agent and a process to produce the same, in particular, a stabilized semi-solid pharmaceutical agent with proteinaceous bioactive substances and a process to produce the same.

To solve the aforementioned object, the present inventors energetically studied semi-solid pharmaceutical agents where saccharides were utilized. As the result, it was found first that offer mixing aqueous solutions containing proteinaceous bioactive substances with a variety of saccharides and comparing the resultant solids for residual activity, the use of oligosaccharides, in particular, those exhibiting a strong

hygroscopicity at a relative humidity of 75%, led to solids which were higher in residual activity ratio in comparison with the case of using monosaccharides, as well as that offer subsequently mixing the resultant solids with a variety of base materials and investigating the resultant for stability of activities, semi-solid pharmaceutical agents with stabilized activity were obtained only when oil or fat bases were used. The present invention is based on this novel finding.

Thereafter the present inventors further investigated optimum combining ratios for these substances and materials, leading to the finding that semi-solids which contained against one part by weight of oligosaccharide about 0.02 or less parts by weight of proteinaceous bioactive substances and about 2 or more parts by weight of oil or fat bases were suitable as semi-solid pharmaceutical agents because they stably retained the activities of proteinaceous bioactive substances and exhibited appropriate extending and shape-retaining abilities. Thus the present inventors completed the invention.

Therefore according to one aspect of the present invention there is provided a semi-solid pharmaceutical composition of a stabilized proteinaceous bioactive substance as an effective ingredient, which comprises a pharmaceutically-acceptable oligosaccharide, a proteinaceous bioactive substance, and an oil and fat base, wherein said proteinaceous bioactive substance is stabilized by said oligosaccharide and said oil and fat base.

According to a further aspect of the present invention there is provided a process to prepare a semi-solid pharmaceutical composition, which comprises mixing an oligosaccharide and a solution of a proteinaceous bioactive substance, and kneading the resultant solid with an oil and fat base.

DETAILED DESCRIPTION OF THE INVENTION

Oligosaccharides feasible in the invention are one or more di- through decasaccharides or their correspondent sugar alcohols which well retain the activities of proteinaceous bioactive substances so that one can produce stabilized semi-solid pharmaceutical agents: For example, disaccharides such

as maltose, trehalose, lactose, sucrose and paratinose; oligosaccharide alcohols such as maltitol, lactitol and maltotriitol; maltooligosaccharides such as maltotriose, panose, maltotetraose and maltopentaose; oligosaccharides having the structure of sucrose within their molecules such as elurose, raffinose, meletitose, lactosylfructoside and maltosylfructoside; and other oligosaccharide mixtures such as "COUPLING SUGAR[®]", a glycosylsucrose commercialized by Hayashibara Co., Ltd., Okayama, Japan, isomaltooligosaccharide, galactooligosaccharide, fructooligosaccharide are arbitrarily used.

Particular, oligosaccharides which notably well stabilize proteinaceous bioactive substances are anhydrous oligosaccharide which exhibits a strong hygroscopicity at a relative humidity of 75%. Such an oligosaccharide is feasible with those in substantially anhydrous form with the possible lowest moisture content, desirably, 3 w/w% or lower and one of the typical oligosaccharide is an anhydrous crystalline maltose product, "FINETOSE[®]", commercialized by Hayashibara Co., Ltd, Okayama, Japan. The moisture contents as referred to in the invention were those as determined by the Karl Fischer's method.

The wording "semi-solid pharmaceutical agent" as referred to in the invention means those in the form of an

ointment, cream, cachou, suppository and the like other than those in the form of a powder and solid.

The wording "proteinaceous bioactive substance" as referred to in the invention means those including simple or complex proteins, more particularly, cytokines, for example, α -, β - and γ -interferons, tumor necrosis factors α and β (TNF- α , TNF- β), epithelial cell growth factor (EGF), transfer factor, T cell growth factor (TCGF), and colony-stimulating factor (CSF), and proteinaceous hormone, for example, such as insulin, growth hormone, prolactin, chorionic gonadotropic hormone, erythropoietin, follicle-stimulating hormone, luteinizing hormone, adrenocorticotrophic hormone, placental lactogen, thyroid-stimulating hormone and parathyroid-stimulating hormone which bear molecular weights up to 5,000-200,000.

Any proteinaceous bioactive substances are feasible in the invention regardless of preparation methods. Proteinaceous bioactive substances which are derived from body fluids, tissues and organs when they are inherently present, those from *in vitro* or *in vivo* cultures thereof and those from cultures of human cells, animal cells and microorganism wherein productibilities of such a substance has been introduced by conventional cell fusion and gene recombinant methods are all feasible in the invention.

In this invention, generally, proteinaceous bioactive

substances are used in aqueous solution form at a concentration of about 5 w/v% or lower, desirably, 1×10^{-5} -3 w/v%.

Generally, one can produce solids such as powder, granule and block where the activities of proteinaceous bioactive substances are stabilized by mixing one part by weight of solid oligosaccharide and about 0.4 or less parts by weight, desirably, 0.001-0.3 parts by weight of an aqueous solution of proteinaceous bioactive substance to a homogeneity as much as possible. If necessary, before completion of the step giving final products, drying step using ventilation drying, vacuum drying and lyophilization and/or sieving step is arbitrarily provided.

To produce objective semi-solid pharmaceutical agent with solids thus obtained, about 2 or more parts by weight against one part by weight of oligosaccharide, desirably, about 3-50 parts by weight of an oil or fat base, for example, white petrolatum, purified lanolin, cacao oil or "PHARMASOL[®]", commercialized by Nippon Oil and Fats Co., Ltd. Thus one can obtain stabilized semi-solid pharmaceutical agents according to the invention which contain one part by weight of oligosaccharide, about 0.02 or less parts by weight of proteinaceous bioactive substance and about 2 or more parts by weight of oil or fat base. If necessary, in the course of producing such a semi-solid pharmaceutical agent, conventional supplements, for



example, stabilizer, absorption-promoting agent, bactericide, filler, taste-imparting agent, coloring agent and flavor-imparting agent can be used in combination so as to improve its efficacy and commercial value.

Semi-solid pharmaceutical agents thus obtained are favorably feasible, after cooling and shaping, in oral agents and a suppositories as well as in ointments in paste form because the use of "PHARMASOL[®]" or cacao oil stabilizes them at 30°C or lower but brings them into melting form around 40°C. These semi-solids are suitable for pharmaceutical agents which are administered through percutaneous or permucosal route. The amount of proteinaceous bioactive substance contained in such a semi-solid pharmaceutical agent can be arbitrarily chosen; for example, in the case of IFN- α , the present agent contains 10^2 - 10^8 IU/g (The wording "IU" means international units.), in the case of IFN- γ , 10 - 10^7 IU/g and in the case of TNF- α , up to 10 - 10^7 IU/g.

The present semi-solid pharmaceutical agent is administered ointment, cachou or suppository form, through the percutaneous and permucosal route, usually, 1-5 times a day in a dose up to 0.01-10g/adult, in order to treat susceptible diseases. These administration route and dose can be arbitrarily changed dependently on the type and content of proteinaceous bioactive substances, as well as on the type of disease

and patient's symptom.

The present invention will be explained in detail hereinafter in conjunction with several EXPERIMENTS:

EXPERIMENT 1

Influence of saccharides on the solidification of proteinaceous bioactive substance

The stabilities of proteinaceous bioactive substances in solid compositions, obtained by mixing to homogeneity aqueous solutions containing proteinaceous bioactive substances with solid saccharides, were examined. In accordance with conventional method, the proteinaceous bioactive substances used in the experiment were prepared by applying an interferon- α (IFN- α) preparation, an interferon- γ (IFN- γ) preparation or a tumor necrosis factor α (TNF- α) preparation, all of which are produced by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, and commercialized by Cosmo Bio Co., Ltd., Tokyo, Japan, to an antibody column fixed with each monoclonal antibody and after removing non-adsorbed fractions, adsorbed fractions were eluted from the column and concentrated by a membrane filter to obtain an about 0.01 w/v% aqueous solution.

The saccharides used in the experiment were monosaccharides such as hydrous crystalline glucose, anhydrous crystalline glucose, anhydrous crystalline fructose and anhydrous crystalline galactose; and oligosaccharides such as

hydrous crystalline maltose, anhydrous crystalline maltose, anhydrous crystalline maltitol, hydrous crystalline trehalose, anhydrous crystalline trehalose, anhydrous crystalline neotrehalose, hydrous crystalline lactose, anhydrous crystalline lactose, anhydrous crystalline sucrose, anhydrous crystalline panose, anhydrous crystalline lactosylglucoside, anhydrous amorphous lactosylfructoside (alias lactosucrose), anhydrous amorphous maltotetraose and anhydrous amorphous glycosylsucrose (COUPLING SUGAR®).

To 50 parts by weight of each oligosaccharide was added one part by weight of an aqueous solution containing one of the proteinaceous bioactive substances prepared by the above method, and mixed as homogeneously as possible while spraying the solution to the oligosaccharide. The resultant mixture was allowed to stand at 30°C for 18 hours and pulverized to obtain a powder containing a proteinaceous bioactive substance. After one-month standing of the resultant powder under drying conditions at 40°C, the residual activity of the proteinaceous bioactive substance was determined. The percentage of the residual activity to the initial activity in the starting aqueous solution was considered as the percentage of the residual activity (%), i.e., the index of the stability of the proteinaceous bioactive substance in the powder. While each saccharide used as a material was determined the level of its

hygroscopicity by allowing it to stand at a relative humidity of 75% and 30° C for 6 days, and determining the final weight. The difference between the final weight and the starting weight was determined and considered as the weight increased by moisture absorption, and the percentage of the increased weight against the starting weight was considered as the ratio of moisture absorption (%), i.e., the index of the level of hygroscopicity. The results were as shown in Table 1.

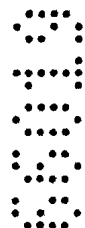


Table 1

Saccharide	Percentage of hygroscopicity(%)	IFN- α	IFN- γ	TNF- α	Judgement
(A)Hydrous crystalline glucose	Less than 1.0	15.1	0	0	Impassable
(A)Anhydrous crystalline glucose	Less than 1.0	25.7	0	0	Impassable
(A)Anhydrous crystalline fructose	Less than 1.0	13.6	0	0	Impassable
(A)Hydrous crystalline galactose	Less than 1.0	12.4	0	0	Impassable
(B)Hydrous crystalline maltose	Less than 1.0	47.2	22.5	9.2	Passable
(B)Anhydrous crystalline maltose	5.9	98.7	56.4	61.1	Excellent
(B)Anhydrous crystalline maltitol	Less than 1.0	49.2	24.8	10.6	Passable
(B)Hydrous crystalline trehalose	Less than 1.0	44.9	31.8	8.0	Passable
(B)Anhydrous crystalline trehalose	9.8	86.1	52.6	57.4	Excellent
(B)Anhydrous crystalline neotrehalose	Less than 1.0	35.4	14.0	7.3	Passable
(B)Hydrous crystalline lactose	Less than 1.0	33.1	2.6	5.4	Passable
(B)Anhydrous crystalline lactose	Less than 1.0	45.4	9.3	9.1	Passable
(B)Anhydrous crystalline sucrose	Less than 1.0	33.5	20.8	8.0	Passable
(B)Anhydrous crystalline panose	Less than 1.0	36.2	14.4	36.5	Passable
(B)Anhydrous crystalline lactosylglucoside	Less than 1.0	32.3	28.6	29.4	Passable
(B)Anhydrous amorphous lactosylfructoside	21.4	98.8	75.6	70.1	Excellent
(B)Anhydrous amorphous maltotetraose	20.3	66.2	47.1	50.3	Excellent
(B)Anhydrous amorphous glycosylsucrose	22.6	80.1	65.9	55.2	Excellent

Note:(A) means monosaccharide, and (B) means oligosaccharide.

These data confirmed that the solidification of a proteinaceous bioactive substance under the coexistence of an oligosaccharide is suitable for the stabilizing the proteinaceous bioactive substance. It is found that oligosaccharides which exert high hygroscopicity of 5% or higher at a relative humidity of 75% and 30°C for 6 days are satisfactorily used, more particularly, anhydrous oligosaccharides such as anhydrous crystalline maltose, anhydrous crystalline trehalose, anhydrous amorphous lactosylfructoside and anhydrous amorphous glycosylsucrose are suitably used.

EXPERIMENT 2

Influence of bases on semi-solidification of proteinaceous bioactive substances solidified by oligosaccharides

A powdery IFN- α stabilized with anhydrous crystalline maltose, a powdery IFN- γ stabilized with anhydrous amorphous lactosylfructoside or a powdery TNF- α stabilized with anhydrous amorphous lactosylfructoside, prepared by the method in EXPERIMENT 1, was kneaded with a semi-solidifying base in usual manner, and the stability of the activity of the proteinaceous bioactive substances in the resultant semi-solid bases were compared.

One part by weight of a powdery proteinaceous bioactive substance was kneaded with 9 parts by weight of a water-soluble base, an emulsion base or an oil or fat base, and allowed to

stand under a relative humidity of 75% and 30°C. After one-month standing, the residual activity of the proteinaceous bioactive substance was determined, followed by calculating the percentage of the residual activity against the starting activity, i.e., the percentage of the residual activity (%) or the index of the stabilization of a proteinaceous bioactive substance in semi-solid pharmaceutical. Furthermore, those prepared with oil or fat bases were heated in phosphate buffer at 40°C for 30 minutes to dissolve and isolate the oil or fat bases. The activity of the proteinaceous bioactive substance in the resultant aqueous solution was determined in usual manner. The results were as shown in Table 2.

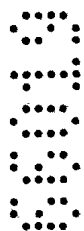


Table 2

Solid Base	coexistence of IFN- α and anhydrous crystalline maltose	coexistence of IFN- γ and anhydrous amorphous lactosylfructoside	coexistence of TNF- α and anhydrous amorphous lactosylfructoside	Judgement
(A)Macrogol Ointment	0	0	0	Impassable
(A)HIVIS WAKO GEL [®]	0	0	0	Impassable
(B)Vanishing Cream	0	0	0	Impassable
(B)Cold Cream	0	0	0	Impassable
(C)White Petrolatum	100	100	100	Passable
(C)PHARMASOL [®]	100	100	100	Passable

Note: Values in the Table 2 mean residual percentages (%) of residual activity. (A) means a water-soluble base, (B) means an emulsion base and (C) means an oil and fat base.

As is obvious from Table 2, it was found that the powders containing proteinaceous bioactive substances which were solidified by oligosaccharides can be made into semi-solid pharmaceuticals containing stabilized proteinaceous bioactive substances, only in the case of mixing the proteinaceous substances with oil or fat bases.

EXPERIMENT 3

Influence of the mixing ratio of powdery proteinaceous bioactive substance and oil or fat base on the formation of semi-solid pharmaceutical

One part by weight of a powdery IFN- α stabilized with anhydrous crystalline maltose, obtained by the method in EXPERIMENT 1, was kneaded with 0.1, 0.5, 1, 2, 5, 10 or 20 parts by weight of white petrolatum or "PHARMASOL[®]", and the resultant mixture was allowed to stand at about 25°C for one hour, followed by the observation of the form of the resultant product. After 6-months standing of the product under a relative humidity of 75% and 30°C, the residual activity of the IFN- α was determined and the percentage of the residual activity against the starting activity was calculated, as the percentage of the residual activity (%), i.e., the index of the stabilization of proteinaceous bioactive substances in the products. For the sake of the comparison, powdery proteinaceous bioactive substances were allowed to stand under

the same condition for 6-months, followed by calculating the percentage of (%) of the residual activity. The results were as shown in Table 3.

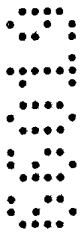


Table 3

Part by weight of an oil and fat base against one part by weight of a solid		Form	Residual activity (%)	Judgement
	0	Powdery	0	Control
(A)	0.1	Nearly powdery	0	Impassable
(A)	0.5	Gel	15.8	Impassable
(A)	1.0	Pasty	64.6	Passable
(A)	2.0	Pasty	100	Excellent
(A)	5.0	Pasty	100	Excellent
(A)	10.0	Pasty	100	Excellent
(A)	20.0	Pasty	100	Excellent
(B)	0.1	Nearly powdery	0	Impassable
(B)	0.5	Gel	20.4	Impassable
(B)	1.0	Solid	73.6	Passable
(B)	2.0	Solid	100	Excellent
(B)	5.0	Solid	100	Excellent
(B)	10.0	Solid	100	Excellent
(B)	20.0	Solid	100	Excellent

Note: (A) means white petrolatum and (B) means PHARMASOL[®].

As is obvious from Table 3, it was found that the suitable mixing ratio of an oil or fat base against one part by weight of a solid containing proteinaceous bioactive substance is about 2 parts by weight, which keeps the resultant product in a desirable form and in a high residual activity.

EXPERIMENT 4

Influence of production condition on the semi-solid pharmaceutical

The influence of materials to be mixed and mixing procedure on the stabilization of proteinaceous bioactive substances in the resultant semi-solid pharmaceuticals was examined.

Sample A was a product which was produced by the representative preparation of the invention, which was prepared by the method in EXPERIMENT 1 by successively mixing homogeneously one part by weight of an aqueous solution containing 0.01 w/v% of IFN- α with 50 parts of weight of anhydrous crystalline maltose into a solid which was then kneaded with white petrolatum by the method in EXPERIMENT 2. The following each sample as a control was prepared by using the same mixing ratio (part by weight) as in sample A. A semi-solid pharmaceutical, sample B, was obtained by successively kneading anhydrous crystalline maltose with white petrolatum and kneading the resultant mixture with an aqueous solution

containing IFN- α as homogeneously as possible.

A semi-solid pharmaceutical, sample C, was obtained by successively kneading anhydrous crystalline maltose with white petrolatum and kneading as homogeneously as possible the resultant substance with a lyophilized product prepared from an aqueous solution containing IFN- α .

A semi-solid pharmaceutical, sample D, was obtained by successively kneading white petrolatum with an aqueous solution containing IFN- α as homogeneously as possible.

A semi-solid pharmaceutical, sample E, was obtained by successively kneading white petrolatum with a lyophilized product prepared from an aqueous solution containing IFN- α .

These samples were allowed to stand under a relative humidity of 75% and 40°C. After one-month standing, the residual activity of IFN- α in each sample was determined and the percentage (%) of the residual activity against the activity in the material aqueous solution was determined as the percentage (%) of the residual activity, i.e., the index of the stabilization of the proteinaceous bioactive substance in the semi-solid pharmaceutical. The results were as shown in Table 4.

Table 4

Sample	Production condition	Residual activity (%)	Judgement
A	(Anhydrous crystalline maltose + Aqueous IFN- α solution) + White petrolatum	100	Present invention
B	(Anhydrous crystalline maltose + White petrolatum) + Aqueous IFN- α solution	4.7	Control
C	(Anhydrous crystalline maltose + White petrolatum) + Dried IFN- α	25.1	Control
D	White petrolatum + Aqueous IFN- α solution	2.7	Control
E	White petrolatum + Dried IFN- α	38.4	Control

As is obvious from Table 4, it was found that the semi-solid pharmaceuticals containing stabilized proteinaceous bioactive substances which were obtained by successively mixing aqueous solutions containing proteinaceous bioactive substances with oligosaccharides and kneading the resultant mixtures with oil or fat bases. It was also found that it is impossible to achieve the present object when oligosaccharides are not used in the materials for the present semi-solid pharmaceutical or when their mixing order is changed.

Several examples of the present invention will hereinafter be described, but do not limit the scope of the present invention:

EXAMPLE 1

Ointment containing IFN- α

Newborn hamsters were injected with antiserum prepared in conventional manner to weaken their immunoreaction, implanted subcutaneously with BALL-1 cells and fed in usual manner for 3 weeks. The tumor masses, formed subcutaneously in the body of the hamsters, were extracted, minced and disaggregated in saline. The cells thus obtained were washed with serum-free RPMI 1640 medium (pH 7.2), suspended in a fresh preparation of the same medium to give a cell density of about 2×10^6 cells/ml, kept at 35°C . The cell suspension was mixed with 200 u/ml of a partially purified IFN- α , kept at this

temperature for 2 hours, mixed with about 300 hemagglutination titer/ml of Sendai virus at this temperature, and incubated for 20 hours to induce IFN- α . The resultant culture was centrifuged to remove insoluble substances at about 1,000 $\times$ g and about 4°C, and the supernatant was concentrated with a membrane filter, and the concentrate was a column of immobilized anti-IFN- α antibody in conventional manner, followed by removing a non-adsorbed fraction. Thereafter, an adsorbed fraction was eluted from the column and concentrated with a membrane filter to obtain an about 0.5 w/v% liquid preparation containing human IFN- α having a specific activity of about 2×10^8 IU/mg protein.

One part by weight of a liquid preparation obtained by the method in EXAMPLE 1 was mixed as homogeneously as possible with 49 parts by weight of anhydrous crystalline maltose to obtain a solid preparation containing IFN- α which was then kneaded with 3-times by volume of white petrolatum to obtain an ointment containing IFN- α . The product had about 5×10^6 IU/g of IFN- α . The product, an ointment containing a stabilized IFN- α , can be advantageously used for treating IFN- α susceptible diseases such as an infectious disease of herpes virus, a cutaneous cancer, a rhinitis and a conjunctivitis by applying it to the skin or the mucous membrane.

EXAMPLE 2

Suppository containing IFN- α

A solid preparation containing human IFN- α , obtained by the method in EXAMPLE 1, was mixed with 9-times by volume of "PHARMASOL[®]" which had been dissolved by heating at 40°C, and the mixture was poured into a mold and cooled to obtain a suppository containing IFN- α . The product had about 2×10^6 IU/g of IFN- α . The product, a suppository containing a stabilized IFN- α , can be advantageously used for treating susceptible diseases of IFN- α such as an infectious disease of herpes virus, a hepatitis and a malignant tumor by administering it into the rectum and the vagina.

EXAMPLE 3

Ointment containing IFN- γ

According to the method in EXPERIMENT 1, a commercially available human IFN- γ preparation was applied to an anti-IFN- γ antibody column in conventional manner. After non-adsorbed fraction was removed, an adsorbed fraction was eluted, and concentrated with a membrane filter to obtain an about 0.5 w/v% solution containing human IFN- γ having a specific activity of about 2×10^7 IU/mg protein.

One part by weight of the solution and 29 parts by weight of anhydrous crystalline trehalose were mixed as homogeneously as possible into a solid product containing IFN- γ , which was then kneaded with 4-times by volume of purified ranolin to obtain an ointment containing IFN- γ .

The product had about 3×10^5 IU/g of IFN- γ . The product, an ointment containing a stabilized IFN- γ , can be advantageously used for treating IFN- γ susceptible diseases such as an infectious disease of virus, a hepatitis, an articular rheumatism, a rthritis and a conjunctivitis by applying it to the skin and the mucous membrane similarly as the product of EXAMPLE 1.

EXAMPLE 4

Suppository containing TNF- α

A non-adsorbed fraction of anti-IFN- α antibody, prepared by the method in EXAMPLE 1, was eluted and concentrated with a membrane filter to obtain an about 0.5 w/v% concentrated solution containing human TNF- α having a specific activity of about 2×10^7 IU/mg protein.

One part by weight of the solution and 49 parts by weight of anhydrous amorphous lactosylfructoside were mixed as homogeneously as possible into a solid product containing TNF- α which was then kneaded with 3-times by volume of "PHARMASOL[®]" to obtain a suppository containing TNF- α .

The product had about 3×10^5 IU/g of TNF- α . The product, a suppository containing a stabilized TNF- α , can be advantageously used for treating TNF- α susceptible diseases such as a malignant tumor, an infectious disease of virus and an immunopathy by administering it into the rectum and the

vagina.

EXAMPLE 5

Cachou containing cytokine

0.5 parts by weight of a solution containing human IFN- α prepared by the method in EXAMPLE 1, 0.25 parts by weight of a solution containing human IFN- γ prepared by the method in EXAMPLE 3, 0.25 parts by weight of a solution containing human TNF- α prepared by the method in EXAMPLE 4 and 9 parts by weight of an aqueous solution containing about 0.5 w/v% bovine serum albumin were mixed, and the mixture and 490 parts by weight of anhydrous crystalline maltose were mixed as homogeneously as possible into a solid product containing cytokine. The solid was mixed with 4-times by volume of cocoa butter previously dissolved by heating, adequate amounts of a coloring agent and a flavor-imparting agent, and the mixture was poured into a mold and cooled to obtain a cachou containing cytokine.

The product, having about 1×10^5 IU/g of IFN- α , about 3×10^3 IU/g of IFN- γ and about 3×10^3 IU/g of TNF- α , is a cachou containing 3 types of stabilized cytokines. Since the efficacy of IFN- α , IFN- γ and TNF- α is exerted synergistically, it is much more effective for treating IFN- α , IFN- γ and TNF- α susceptible diseases such as an immunopathy, an infectious disease of virus and a malignant tumor by administering it to the mouth.

EXAMPLE 6

Suppository containing cytokine

A mixture solution containing cytokine, which was prepared by the method in EXAMPLE 5, and 490 parts by weight of anhydrous crystalline trehalose were mixed as homogeneously as possible to obtain a solid containing cytokine which was then poured into a mold and cooled to obtain a suppository containing cytokine.

The product, having about 4×10^4 IU/g of IFN- α , about 1×10^3 IU/g of IFN- γ and about 1×10^3 IU/g of TNF- α , is a suppository containing 3 types of stabilized cytokines. Since, the efficacy of IFN- α , IFN- γ and TNF- α is exerted synergistically, it is much more effective for treating IFN- α , IFN- γ and TNF- α susceptible diseases such as an immunopathy, an infectious disease of virus and a malignant tumor by administering it into the rectum and the vagina.

EXAMPLE 7

Ointment containing EGF

0.0001 part by weight of a commercially available recombinant human EGF was dissolved in one part by weight of phosphate-buffered saline (pH 7.2), and the resultant solution was mixed with 49 parts by weight of anhydrous crystalline maltose as homogeneously as possible to obtain a solid. The resultant solid was kneaded with 9-times by volume of white

petrolatum to obtain an ointment containing EGF.

The product had about 0.2 µg/g of active EGF. The product, an ointment containing a stabilized EGF, can be advantageously used for treating EGF susceptible diseases such as a trauma, an incisure, an abrasion and a burn by applying it to the skin or the mucous membrane to effectively treat them.

EXAMPLE 8

Oral agent containing insulin

0.1 part by weight of a commercially available recombinant human insulin having a specific activity of about 24 IU/mg protein was dissolved in one part by weight of phosphate-buffered saline (pH 7.0), and the resultant solution was mixed with 49 parts by weight of anhydrous crystalline maltose as homogeneously as possible into a solid. The resultant solid was kneaded with 19-times by volume of cocoa butter, which had been previously dissolved by heating, and an adequate amount of a flavor-imparting agent, and the mixture was poured into a mold and cooled to obtain an oral agent containing insulin.

The product had about 21 IU/g of insulin. The product, an oral agent containing a stabilized insulin, can be advantageously used for keeping the blood sugar level of diabetics within a normal level by administering them about 2-6

IU/day of insulin.

EXAMPLE 9

Suppository containing erythropoietin

0.01 part by weight of a commercially available human erythropoietin derived from urine having a specific activity of about 1×10^5 IU/mg protein was dissolved in phosphate-buffered saline (pH 7.2), and the resultant solution was mixed with 49 parts by weight of anhydrous crystalline trehalose as homogeneously as possible to obtain a solid. The resultant solid was mixed with 39-times by volume of "PHARMASOL[®]" which had been previously dissolved by heating, and the mixture was poured into a mold and cooled to obtain a suppository containing erythropoietin.

The product has about 500 IU/g of erythropoietin. The product, a suppository containing a stabilized erythropoietin, can be advantageously used for keeping the number of erythrocyte for anemic patients by administering them about 1,000-6,000 IU/day of erythropoietin.

[Effect of the invention]

As described above, the present invention established semi-solid pharmaceuticals containing stabilized proteinaceous bioactive substances in the form of an ointment and a suppository. The preparations comprise mixing solutions containing proteinaceous bioactive substances with

oligosaccharides to obtain solid products, and kneading the resultant solid products with oil or fat bases.

Proteinaceous bioactive substances contained in the semi-solid pharmaceuticals are effective for treating susceptible diseases, and the oil or fat bases protect the skin and the mucous membrane. The oligosaccharides in the semi-solid pharmaceuticals attain more satisfactory therapeutic efficacy to supplement energy to cells and cellular tissues. The present semi-solid pharmaceutical readily handles because it is readily administered to the body by percutaneous and permucosal routes which are safer and less in pain administration routes than conventional administrations.

The claims defining the invention are as follows:

1. A semi-solid pharmaceutical composition in the form of an ointment, cachou or suppository which is administered to a subject through a percutaneous or permucosal route, said composition comprising a pharmaceutically-acceptable oligosaccharide, a proteinaceous bioactive substance as an effective ingredient for medical use, and a pharmaceutically-acceptable oil and fat base, wherein said proteinaceous bioactive substance is stabilised by said oligosaccharide and said oil and fat base, and said composition containing with respect to one part by weight of said oligosaccharide, 0.02 or lower part by weight of said proteinaceous bioactive substance and 2 or higher parts by weight of said oil and fat base.
2. The semi-solid pharmaceutical composition in accordance with claim 1, wherein said proteinaceous bioactive substance is a member selected from the group consisting of cytokines, hormones, and mixtures thereof.
3. The semi-solid pharmaceutical composition in accordance with claim 1 or claim 2, wherein said oligosaccharide is a member selected from the group consisting of di- through deca-saccharides, their sugar alcohols, and mixtures thereof.
4. The semi-solid pharmaceutical composition in accordance with any one of claims 1 to 3, wherein said oligosaccharide is one which exhibits a strong hygroscopicity at a relative humidity of 75%.
5. The semi-solid pharmaceutical composition in accordance with any one of claims 1 to 4, wherein said oil and fat base is a member selected from the group consisting of white petrolatum, lanolin, cacao oil, PHARMASOL®, and mixtures thereof.
6. A process to prepare a semi-solid pharmaceutical composition, which comprises mixing an oligosaccharide and a solution of a proteinaceous bioactive substance, and kneading the resultant solid with an oil and fat base.
7. The process in accordance with claim 6, wherein 0.4 or lower parts by weight of said aqueous solution and 2 or higher parts by weight of said oil and fat base are incorporated into one part by weight of said oligosaccharide.
8. The process in accordance with claim 6 or claim 7, wherein said solution

is an aqueous solution.

9. The process in accordance with any one of claims 6 to 8, wherein said proteinaceous bioactive substance is a member selected from the group consisting of cytokines, hormones, and mixtures thereof.

5 10. The process in accordance with any one of claims 6 to 9, wherein said aqueous solution contains at most about 5 w/v% of said proteinaceous bioactive substance.

10 11. The process in accordance with any one of claims 6 to 10, wherein said oligosaccharide is a member selected from the group consisting of di- through deca-saccharides, their sugar alcohols, and mixtures thereof.

12. The process in accordance with any one of claims 6 to 11, wherein said oligosaccharide is one which exhibits strong hygroscopicity at a relative humidity of 75%.

15 13. The process in accordance with any one of claims 6 to 12, wherein said oil and fat base is a member selected from the group consisting of white petrolatum, lanolin, cacao oil, PHARMASOL®, and mixtures thereof.

14. The process in accordance with any one of claims 6 to 13, wherein said semi-solid pharmaceutical composition is in the form of an ointment, a cachou or a suppository.

20 15. The semi-solid pharmaceutical composition in accordance with any one of claims 1 to 5, substantially as hereinbefore described with reference to any one of the examples.

16. The process in accordance with any one of claims 6 to 14, substantially as hereinbefore described with reference to any one of the examples.

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ABSTRACT OF THE DISCLOSURE

Disclosed is a semi-solid pharmaceutical agent containing a stabilized proteinaceous bioactive substance prepared by successively mixing an oligosaccharide, and an aqueous solution of a proteinaceous bioactive substance, and kneading the resultant solids with an oil or fat base. The pharmaceutical handles with ease because it is readily administered to the body through percutaneous and permucosal route which are safer and less in pain administration routes than other conventional administrations.