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(54) Films/sheets of hyaluronic acid

(57) A method for producing a readily water-soluble film or sheet of hyaluronic acid or a hyaluronate, comprises forming a layer of an aqueous solution of hyaluronic acid or a hyaluronate and freeze-drying the layer in vacuo. A readily water-soluble cosmetic sheet, prepared by the preceding method, comprises hyaluronic acid or a hyaluronate together with magnesium-L-ascorbil phosphate.

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SHEETS COMPRISING HYALURONIC ACID, THE PRODUCTION AND THE USE OF THE SHEETS

The present invention relates to easily water-soluble sheets principally comprising hyaluronic acid, the production and the use as cosmetic.

Hitherto, as a so-called one-point cosmetic giving moderate moisture and tension to the skin by applying the cosmetic to a part of eyelids, the corner of the eye, a forehead, lips, a neck and the like, a film made of a collagen hydrolysate or a material obtained by immersing the collagen hydrolysate in a nonwoven sheet and drying the sheet was used.

Since hyaluronic acid or hyaluronate (abbreviated as hyaluronic acid (hyaluronate) hereinafter) has excellent properties as a moisturizer, skin cells are activated, aged skin is rapidly turned over, and chapped skin and horny substances are remarkably improved. As a result, it is said that the compound gives moisture, smooth, tension and gloss to the skin and it has an effect to make the skin beautiful.

Magnesium-L-ascorbil phosphate has stability in air and water, and it is absorbed through the skin in a living body and easily hydrolyzed by an enzyme such as phosphatase or the like to give L-ascorbic acid which exerts whitening effect to the skin.

It is expected to use a mixture of hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate having such effects as a one-point cosmetic which gives moderate moisture and tension to the skin by applying to a part of eyelids, the corner of the eye, a forehead, lips, a neck and the like on which wrinkles easily appear.

However, the dried material of hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate is powder. It is difficult to dissolve a sheet or a film

obtained by drying an aqueous solution of hyaluronic acid (hyaluronate) or an aqueous solution of a mixture of hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate in water or toilet water. Accordingly, it is impossible to use the sheet or film as a one-point cosmetic.

The object of the present invention is to provide a method for producing a sheet or a film having good solubilities in water or toilet water by using hyaluronic acid (hyaluronate) which has excellent merits as described above.

Further, the other object of the present invention is to provide a one-point cosmetic having excellent merits, such as improvement of chapped skin and horny substances, rapid turnover of horny substance layers, give of beautiful skin and the like for prevention of skin aging.

Moreover, the object of the present invention is to provide a cosmetic sheet or film for using as one-point cosmetics giving moderate moisture and tension to the skin and whitening the skin.

Further, the object of the present invention is to provide a method for producing a cosmetic sheet or a cosmetic film having good solubilities in water or toilet water by using hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate which have excellent beauty merits as described above.

The present invention provides a method for producing an easily water-soluble, particularly an easily toilet water-soluble sheet of hyaluronic acid (hyaluronate) characterized in that it comprises sheeting an aqueous solution of hyaluronic acid (hyaluronate) and freeze-drying the obtained sheet in vacuo. Further, the present invention provides a cosmetic, which is preferably able to use as a one-point cosmetic, having excellent properties for giving beauty skin, comprising a sheet of hyalu-

ronic acid or hyaluronate which is produced by said method.

Further, the present invention provides an easily water-soluble cosmetic sheet, preferably a one-point cosmetic having excellent beauty effect, comprising hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate. Further, the present invention provides a method for producing an easily water-soluble sheet characterized in that it comprises sheeting an aqueous solution obtained by dissolving hyaluronic acid or hyaluronate and magnesium-L-ascorbil phosphate in water, and freeze-drying in vacuo the sheet obtained.

Hyaluronic acid used in the present invention is found in animal tissue, e. g. in joints, vitreous humor, umbilical cords, cartilage, the skin, rooster combs and it plays an important part in living bodies. Sodium hyaluronate is a high-molecular substance. Since the solution of sodium hyaluronate has high viscosity, elasticity and water retention, it is widely used as cosmetic materials, ophthalmology medicine, eyewater and arthropathy medicine.

Hitherto, the production of hyaluronic acid (hyaluronate) is industrially conducted by extracting from rooster combs, glass bodies of cow's eyes, umbilical cords and the like, or by incubating micro-organisms having the ability of hyaluronic acid production (a fermentation method). Hyaluronic acid (hyaluronate) used in the present invention can be obtained either by the extraction method or by the fermentation method.

Hyaluronic acid (hyaluronate) having a molecular weight from 50,000 to 3,000,000 is industrially produced. Hyaluronic acid (hyaluronate) having said molecular weight range can be used in the present invention.

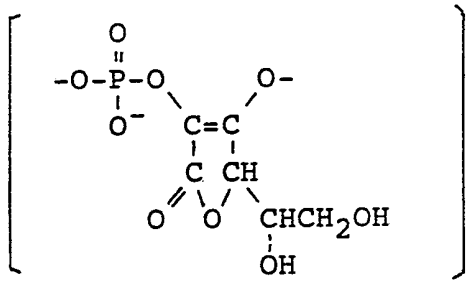
L-ascorbic acid (Vitamin C) is broadly distributed in the vegetable kingdom and the animal kingdom, and partic-

ularly it is richly contained in green vegetables and fruits. It is known that L-ascorbic acid has various kinds of physiological and pharmacological effect, and particularly, the treatment effect for pigmentation trouble is known among the persons concerned in the industry of cosmetics. The most important factor for determining skin colors is melanin. L-ascorbic acid exerts the following effect to melanin.

1) Dopaquinone which is produced in the initial stage of melanin formation is reduced by using L-ascorbic acid to DOPA.

2) Melanin is reduced and turns into a light color type.

On one hand, L-ascorbic acid is relatively stable on drying, on the other hand, it has a disadvantage that it is easily oxidized in an aqueous solution in the presence of air and light. Accordingly, derivatives of L-ascorbic acid having water-solubility and stability in air and water and easily blended in cosmetic materials have been investigated. As a result, magnesium-L-ascorbil phosphate represented by the following formula has been prepared as cosmetics for whitening the skin. In the present invention, the above magnesium-L-ascorbil phosphate is used.



3/2 Mg

For sheeting and freeze-drying an aqueous solution containing hyaluronic acid (hyaluronate), the following process is carried out as an example. Firstly, hyaluronic acid (hyaluronate) is slowly added with stirring to water so as to dissolve thoroughly without lumps and to obtain

an aqueous solution containing 0.05% - 2.0% by weight of hyaluronic acid (hyaluronate). A kind of water for preparing the aqueous solution is not limited particularly, but water used for preparing common cosmetics, for example, ion exchange water or distilled water can be used. Further, toilet water can be used. The hyaluronic acid (hyaluronate) aqueous solution obtained is poured in a shallow vessel having a flat bottom. When the viscous aqueous solution of hyaluronic acid (hyaluronate) forms plane surface, it is cooled and prefrozen. Then, the prefrozen product is put in a vacuum freeze-drying apparatus and freeze-dried in high vacuum. The freeze-dried product is taken out of the vessel to obtain a white sheet (a thin layer) of hyaluronic acid (hyaluronate).

Then, the freeze-drying of the thin layer of the aqueous solution obtained by dissolving hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate is carried out by the following process. Firstly, hyaluronic acid (hyaluronate) is slowly added with stirring to water so as to dissolve thoroughly. To the obtained aqueous solution containing 0.05%-2.0% by weight of hyaluronic acid (hyaluronate), magnesium-L-ascorbil phosphate is added and stirred to dissolve thoroughly. Since the solubility of magnesium-L-ascorbil phosphate is 15-20% by weight, the compound is added to contain preferably 0.05 - 15% by weight in the aqueous solution. Otherwise, hyaluronic acid (hyaluronate) may be added to an aqueous concentrate containing magnesium-L-ascorbil phosphate. In the present invention, the weight ratio of hyaluronic acid (hyaluronate) to magnesium-L-ascorbil phosphate can be arbitrarily changed. Practically, the weight ratio is preferably 0.1-50 of magnesium-L-ascorbil phosphate to hyaluronic acid (hyaluronate). The aqueous solution containing a mixture of hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate is poured in a shallow

vessel having a flat bottom. When the viscous aqueous solution containing a mixture forms plane surface, it is cooled and prefrozen. The temperature for prefreezing the solution is preferably -20 to -40°C. The prefrozen product is put in a vacuum freeze-drying apparatus and freeze-dried under high vacuum. The shelf temperature for freeze-drying is preferably from the normal temperatures to -20°C and the degree of vacuum is preferably 1 torr or less. The product can be warmed in the vacuum freeze drying process. The freeze-dried product is taken out of the vessel to obtain a white sheet (a thin layer) of a mixture of hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate.

The prefreezing can be conducted in the vacuum freeze-drying apparatus. In this case, successive operation from prefreezing to freeze-drying can be carried out in the vacuum freeze-drying apparatus.

Lately, considering the safety of cosmetics, efforts are made to decrease bacterium in products. When a sheet having few bacterium is produced, hyaluronic acid (hyaluronate) or an aqueous solution of hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate is filtered to remove the bacterium with a filter having a hole diameter of about 0.45 μm. The filtrate is freeze-dried in a clean vacuum freeze-drying apparatus, so that the sheet having few bacterium can be obtained.

Since the freeze-dried product prepared by a dilute aqueous solution tends to become a sheet having a very little bulk density, the physical strength of the sheet obtained is very weak. Accordingly, to obtain a sheet having a practical physical strength, an aqueous solution having a concentration of about 0.1% by weight or more is preferably freeze-dried. When a viscous aqueous solution of a mixture of hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate is freeze-dried, it is

preferably prefreeze-dried at a temperature of about -40°C.

In the present invention, the thickness of the sheet can be arbitrarily prepared by regulating the depth of the liquid layer. The thickness of about 0.5 - 3.0 mm is practical in cosmetics.

When the aqueous solution is poured in a shallow vessel having a flat bottom, a piece of release paper surface-treated with silicone resin is put on the bottom of the vessel, by which the dried sheet of hyaluronic acid (hyaluronate) can be smoothly taken out.

The sheet is preferably freeze-dried at about 0.05 torr under high vacuum.

In the production of the sheet of the present invention, surfactants, fillers, pigments, colorants, chelating agents, antioxidants, ultraviolet absorbing agents, perfumes and the like can be added to the aqueous solution of hyaluronic acid (hyaluronate) or the aqueous solution of hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate.

The sheet obtained by the method of the present invention is characterized in that it is easily dissolved in water or face lotion. Firstly, a part of the corner of the eye or the like is moistened with face lotion. Then the cosmetic sheet of the present invention which is cut in a proper size or shape such as a tear drop, a semicircle, an oval or the like is stuck on said part. The sheet is rapidly dissolved in the face lotion to become a viscous solution. When the face lotion or water is insufficient to dissolve the sheet, a proper quantity of water or face lotion can be added to wet and dissolve the sheet thoroughly. The other hand, firstly, the cosmetic sheet of the present invention which is cut in the tear drop shape or the like is stuck on a part of the corner of the eye or the like, and then the sheet is moistened with

water or face lotion. When the sheet moistened with water or face lotion is lightly covered by a finger, the sheet can be easily dissolved in water or face lotion. After a certain time, the face is washed with water, and the hyaluronic acid (hyaluronate) or the mixture of the hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate is dissolved in water to remove from the skin.

The cosmetic sheet obtained by the method of the present invention can be used as cosmetics not only on a part of the corner of the eye but also on a part of a forehead, lips, a neck or the like where wrinkles are easily found. The sheet can be also used on the other parts. To the part of the skin on which the sheet is used as a cosmetic, moderate moisture and tension is given by the beauty effect of hyaluronic acid (hyaluronate). Further, the skin is whitened by the whitening effect of magnesium-L-ascorbil phosphate.

According to the present invention, a cosmetic sheet of a hyaluronic acid (hyaluronate) or a mixture of hyaluronic acid (hyaluronate) and magnesium-L-ascorbil phosphate easily dissolved in water or face lotion can be produced. By using the sheet principally comprising them, a one-point cosmetic having excellent properties can be produced, for prevention of skin aging process, for moisturizing a rough skin, for improving horny substances, for advancing the turn over of horny substance layers, for giving a beauty skin, etc..

The following Examples illustrate the present invention more specifically, but these will not always be precise in practical applications.

Example 1

In 100 ml of purified water, 0.50 g of hyaluronic acid (molecular weight: 1,200,000) was dissolved. The solution obtained was filtered through a filter having a hole diameter of 0.45 μm. The filtrate was poured in an

aluminum vat to obtain a thickness of 2 mm, and prefrozen in a freeze-drying apparatus (manufactured by Loveconco company, a stoppering tray drier) at -40°C. After the prefreezing, the product was freeze-dried for 20 hours at a degree of vacuum of 0.05 torr to obtain 0.42 g of a sheet of hyaluronic acid. The sheet was cut in rectangular pieces having a length of 3 cm and a width of 4 cm. A piece of the sheet having a weight of 9.6 mg was used as a cosmetic.

The pieces of the cosmetic sheet were put in a laboratory dish in which water was thinly spread and they were dissolved in a very short time by absorbing the water.

Comparative Example 1

In 100 ml of purified water, 0.50 g of hyaluronic acid (molecular weight: 1,200,000) was dissolved. The solution obtained was filtered through a filter having a hole diameter of 0.45 μm. The filtrate was poured in an aluminum vat to obtain a thickness of 2 mm, and dried for 8 hours at a temperature of 60°C in a thermostatic dryer. 0.45 g of a film of hyaluronic acid was obtained. The film was cut in rectangular pieces having a length of 3 cm and a width of 4 cm.

The pieces were put in a laboratory dish in which water was thinly spread, however, it was not dissolved but swollen after 10 minutes.

Example 2

In 150 liters of purified water, 0.30 kg of sodium hyaluronate (molecular weight: 1,200,000) produced by a fermentation method was dissolved. 2.0 kg of mannitol was added to the solution of sodium hyaluronate and dissolved thoroughly by stirring. The solution obtained was filtered through a filter having a hole diameter of 0.45 μm. The filtrate was poured in an aluminum vat to obtain a thickness of 2 mm, and prefrozen in a freeze-drying

apparatus (manufactured by KYOWA company, a vacuum freeze-drying apparatus) at -50°C. After the prefreezing, the product was freeze-dried for 2 days at a vacuum degree of 0.05 torr to obtain 2.21 kg of a sheet of sodium hyaluronate. The sheet was cut in rectangular pieces having a length of 3 cm and a width of 4 cm. The pieces were used as a cosmetic sheet.

The pieces of the cosmetic sheet were put in a laboratory dish in which water was thinly spread and they were dissolved in a very short time by absorbing the water.

Comparative Example 2

The filtrate of the mixture of sodium hyaluronate and mannitol same as used in Example 2 was poured in an aluminum vat to obtain a thickness of 2 mm, and dried for 8 hours at a temperature of 60°C in a thermostatic dryer. 2.40 kg of a film of sodium hyaluronate was obtained. The film was cut in rectangular pieces having a length of 3 cm and a width of 4 cm.

The pieces were put in a laboratory dish in which water was thinly spread, however, all of them were not dissolved after 10 minutes.

Example 3

In 100 ml of purified water, 0.50 g of hyaluronic acid (molecular weight: 1,200,000) was dissolved. To the solution, 1.0 g of magnesium-L-ascorbil phosphate was added by stirring to dissolve thoroughly. The mixture was filtered through a filter having a hole diameter of 0.45 μm. The filtrate was poured in an aluminum vat to obtain a thickness of 2 mm, and prefrozen in a freeze-drying apparatus (manufactured by Loveconco company, a stoppering tray drier) at -40°C. After the prefreezing, the product was freeze-dried for 20 hours at a degree of vacuum of 0.05 torr to obtain 1.32 g of a sheet of a mixture of hyaluronic acid and magnesium-L-ascorbil

phosphate. The sheet was cut in rectangular pieces having a length of 3 cm and a width of 4 cm. A piece of the sheet having a weight of 32 mg was used as a cosmetic.

The pieces of the cosmetic sheet were put in a laboratory dish in which water was thinly spread and they were dissolved in a very short time by absorbing the water.

Comparative Example 3

In 100 ml of purified water, 0.50 g of hyaluronic acid (molecular weight: 1,200,000) was dissolved. To the solution obtained, 1.0 g of magnesium-L-ascorbil phosphate was added by stirring to dissolve thoroughly. The mixture was filtered through a filter having a hole diameter of 0.45 μm. The filtrate was poured in an aluminum vat to obtain a thickness of 2 mm, and dried for 16 hours at a temperature of 60°C in a thermostatic dryer. 1.40 g of a film of a mixture of hyaluronic acid and magnesium-L-ascorbil phosphate was obtained. The film was cut in rectangular pieces having a length of 3 cm and a width of 4 cm.

The pieces were put in a laboratory dish in which water was thinly spread, however, it was not dissolved but swollen after 10 minutes.

Example 4

In 5 liters of purified water, 10 g of sodium hyaluronate (molecular weight: 1,200,000) produced by a fermentation method was dissolved. 190 g of magnesium-L-ascorbil phosphate was added to the solution of sodium hyaluronate and dissolved thoroughly by stirring. The solution obtained was filtered through a filter having a hole diameter of 0.45 μm. The filtrate was poured in an aluminum vat to obtain a thickness of 2 mm, and prefrozen in a freeze-drying apparatus (manufactured by KYOWA company, a vacuum freeze-drying apparatus) at -50°C. After the prefreezing, the product was freeze-dried for 2

days at a vacuum degree of 0.05 torr to obtain 180 g of a sheet of a mixture of sodium hyaluronate and magnesium-L-ascorbil phosphate. The sheet was cut in rectangular pieces having a length of 3 cm and a width of 4 cm. The pieces were used as a cosmetic sheet.

The pieces of the cosmetic sheet were put in a laboratory dish in which water was thinly spread and they were dissolved in a very short time by absorbing the water.

Comparative Example 4

The filtrate of the mixture of sodium hyaluronate and magnesium-L-ascorbil phosphate same as used in Example 4 was poured in an aluminum vat to obtain a thickness of 2 mm, and dried for 8 hours at a temperature of 60°C in a thermostatic vacuum dryer. 5.4 kg of a film of a mixture of sodium hyaluronate and magnesium-L-ascorbil phosphate was obtained. The film was cut in rectangular pieces having a length of 3 cm and a width of 4 cm.

The pieces were put in a laboratory dish in which water was thinly spread, however, all of them were not dissolved after 10 minutes.

Example 5

A cosmetic comprising sodium hyaluronate and a cosmetic comprising the sheet of a mixture of sodium hyaluronate and magnesium-L-ascorbil phosphate of the present invention was tested. A panel of 10 women with dark complexion was tested. A cosmetic sheet of sodium hyaluronate obtained in Example 1 and a cosmetic sheet of a mixture of sodium hyaluronate and magnesium-L-ascorbil phosphate obtained in Example 4 was taken the form of the mask hollowing out parts of eyes, a nose and a mouth.

In the test, a proper amount of face lotion having a constitution as shown in Table 1 was applied on a part of face every night after washing their faces. Then, a piece of the cosmetic sheet was put on said part. When the face

lotion is insufficient to dissolve the sheet piece, a proper quantity of the face lotion can be added on the sheet piece and the sheet piece was dissolved in the face lotion by lightly covering by a finger.

After it was maintained overnight as it was, the face lotion was removed by washing their faces.

Effectiveness of the product was tested for two months and the panels were judged themselves about 3 items as shown in Table 2. The results are shown in Table 2 (a cosmetic comprising sodium hyaluronate) and Table 3 (a cosmetic comprising the sheet of a mixture of sodium hyaluronate and magnesium-L-ascorbil phosphate).

Table 1

Ingredients in cosmetic lotion	% by weight
Ethanol	8.0
Propylene glycol	4.0
Glycerin	3.0
Polyoxyethylene oleyl alcohol ether	1.0
Sodium citrate	0.1
Methyl p-oxybenzoate	0.1
Ion exchange water	residue

Table 2

	Results of judgment		
	effective	a little effective	ineffective
Skin moisture	8	2	0
Skin tension	7	3	0
Skin moisture in the next morning	6	3	1

As shown in Table 2, skin moisture and skin tension

are recognized by the cosmetic sheet of sodium hyaluronate of the present invention.

Table 3

	Results of judgment		
	effective	a little effective	ineffective
Skin moisture	8	2	0
Skin tension	7	3	0
Whitening effect	6	2	2

As shown in Table 3, skin moisture, skin tension and whitening effect are recognized by the cosmetic sheet of a mixture of sodium hyaluronate and magnesium-L-ascorbil phosphate of the present invention.

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Claims:

1. A method for producing an easily water-soluble sheet of hyaluronic acid or a hyaluronate, which comprises forming a layer of aqueous solution of hyaluronic acid or a hyaluronate and freeze-drying the layer in vacuo.
2. A method as claimed in claim 1, wherein the aqueous solution comprises toilet water.
3. An easily water-soluble cosmetic sheet when obtained by the method of claim 1.
4. An easily water-soluble cosmetic sheet, which comprises hyaluronic acid or a hyaluronate, together with magnesium-L-ascorbyl phosphate.
5. A cosmetic sheet as claimed in claim 4, which comprises 0.1 - 50 parts by weight of magnesium-L-ascorbyl phosphate per part by weight of hyaluronic acid or hyaluronate.
6. A method for producing an easily water-soluble cosmetic sheet which comprises forming a layer of an aqueous solution comprising hyaluronic acid or a hyaluronate together with magnesium-L-ascorbil phosphate and freeze-drying the layer in vacuo.
7. A method as claimed in claim 6, wherein the aqueous solution is obtained by dissolving 1 part by weight of hyaluronic acid or hyaluronate and 0.1 - 50 parts by weight of magnesium-L-ascorbyl phosphate in water.
8. A method as claimed in claim 6 or claim 7 wherein the aqueous solution comprises toilet water.

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