

Phyto Hyaluronic Acid - WSK

TREMELLA FUCIFORMIS POLYSACCHARIDE



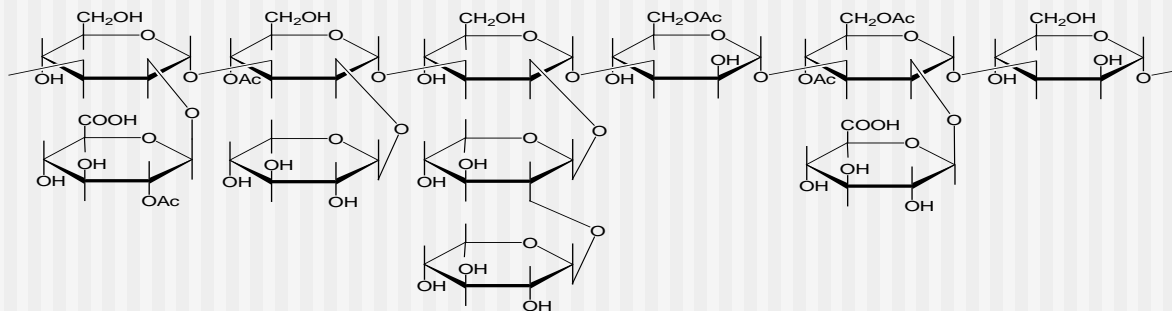
Tremella fuciformis Polysaccharide is an acidic hetero-polysaccharide extracted from the edible fruit bodies of the mushroom called Silver Ear in China. It is said that imperial Concubine Yang Kuei-fei, considered to be the most beautiful woman in Chinese history, used it for her facial and body care.

You can utilize its high moisturizing property, its excellent skin-care effect and its good feel for your formulations.



上海辉文生物技术有限公司
Shanghai Huiwen Biotech Co., Ltd.

Chemical structure and INCI name



Constituent sugar : Mannose, Xylose, Glucuronic acid

Average molecular weight : > 1,000,000

INCI name : Tremella Fuciformis Sporocarp Extract

CTFA Trade name: Tremella Hyaluronic Acid

CAS No.: 778577-37-0

This material is a water soluble high polymer having its origin in **natural vegetable** and with high molecular weight over 1,000,000.

This polysaccharide is constructed from mannan main chain with many short branches. Constituent sugars of the branches are xylose and glucuronic acid.

WSK is a kind of ‘**plant-derived hyaluronic acid**’, because it contains much amount of **glucuronic acid** (about 20 %) as hyaluronic acid does.

Characteristics of WSK

- * This polysaccharide has extremely **high moisturizing effect**.
- * **WSK** produces excellent skin-care effect. **Anti-oxidation effect** was shown by an *in vitro* experimental system using skin cells.
- * Aqueous solution of this polysaccharide has high viscosity, but does **not** show **sticky feel**.
- * The viscosity of the aqueous solution shows **high acid, alkaline and salinity resistance**.
- * For **Skincare** Products, WSK exhibits good **affinity** for skin and makes skin **moist** and **smooth**.
- * For **Haircare** Products---gives **supple** and **smooth** feel .

Capacity for Holding Water

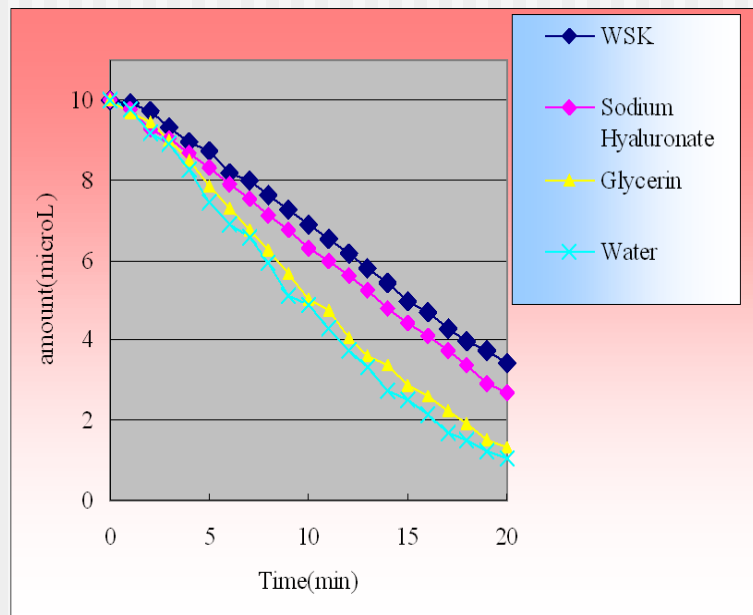
-- WSK retains moisture tightly --

Evaporation of moisture from a WSK aqueous solution was significantly slow compared to other aqueous solutions ($P < 0.01$). WSK exceeds hyaluronic acid in water holding capacity.

<Experimental>

Each 0.2 % aqueous soln. was applied uniformly to a filter paper.

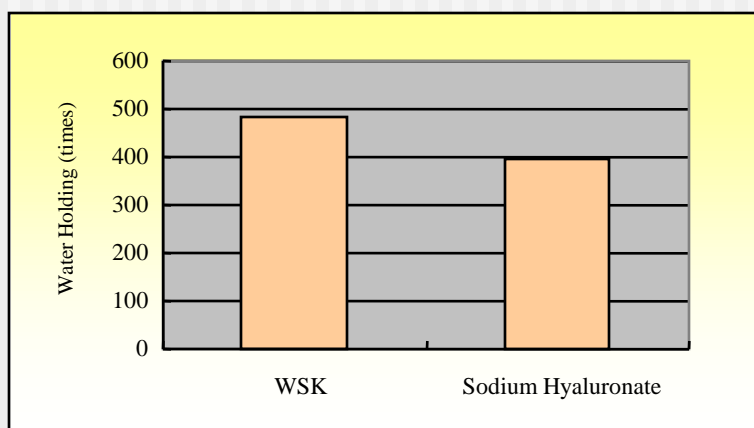
Weight of the filter paper was measured with time under the fixed conditions (25 degrees C, 50 % RH).



Capacity for Holding Water

<Experimental>

0.2 g of powdery specimen was dispersed into 100 g of distilled water. After leaving 2 hrs. at rest, the aqueous soln. was filtrated through No. 2 filter paper, and the weight of the gel on the filter paper was measured.



Capacity for Holding Water

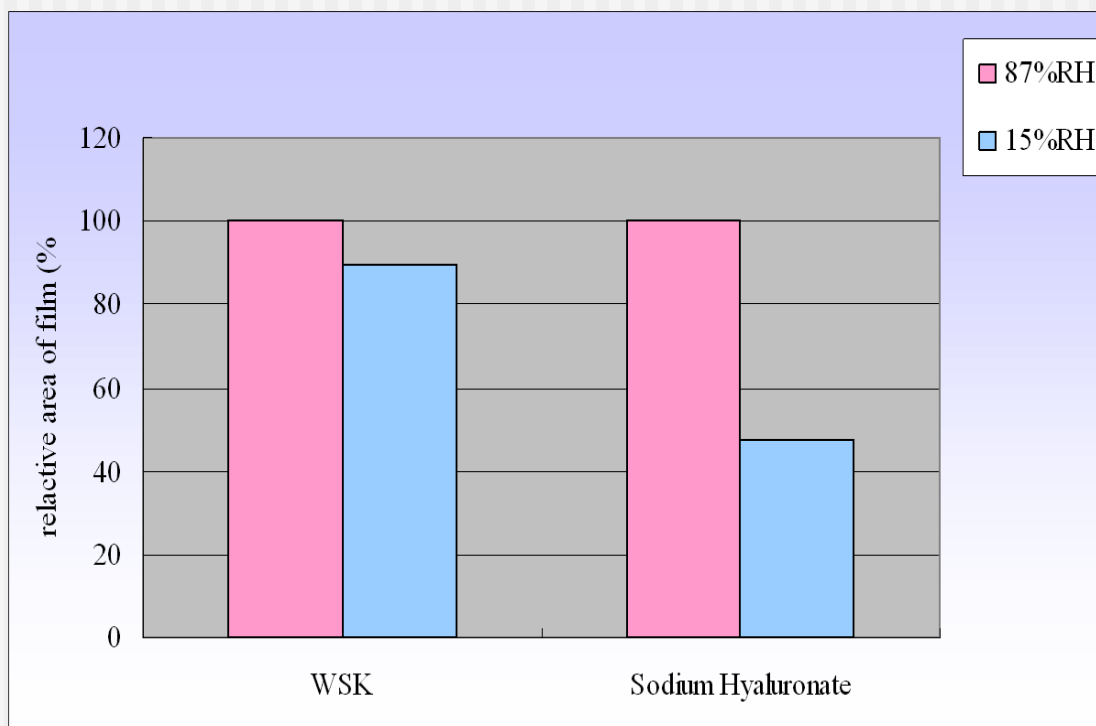
Polysaccharide film Shrinkage Test (1)

-- Not shrink, so Not feel taut --

WSK's film doesn't shrink a lot with humidity changes,
so you don't feel taut.

<Experimental>

The film (15mm x15mm) of **WSK** and that of Sodium Hyaluronate, which are prepared by drying each polysaccharide solution on petri dishes, were kept on the Teflon board under the condition of 25 degree-C/87%RH for 24 hours, and they were transferred and kept under the condition of 25 degree-C/15%RH for 8 hours, then the changes in the film area were measured.



Polysaccharide film Shrinkage Test

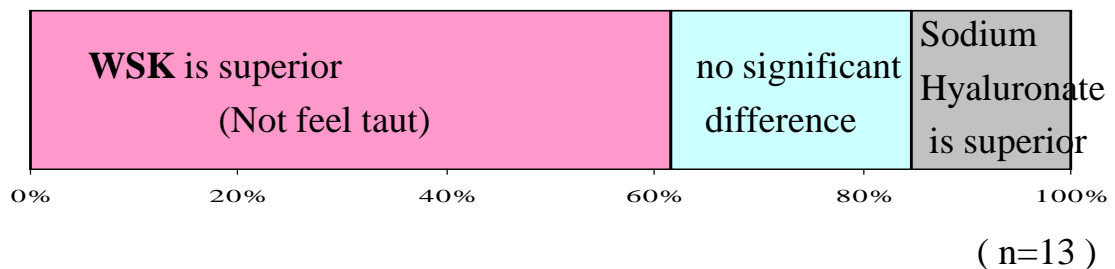
Polysaccharide film Shrinkage Test (2)

-- Not feel taut --

You don't feel taut when the solution of WSK was applied on your face and dried.

<Experimental>

0.2% solution of **WSK** and that of **Sodium Hyaluronate** were applied on each side face, then sensory evaluation was done in blind test.



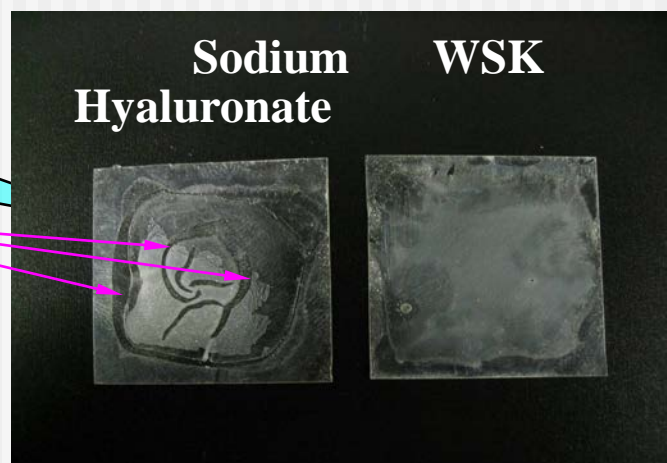
Shrinkage of the film

While many cracks were formed on the film of Sodium Hyaluronate, very few cracks were formed on the film of WSK, and the film of WSK was much more flexible than that of Sodium Hyaluronate.

<Experimental>

Each 1% solution (0.5g) was applied on the OHP sheet(30mm x 30mm square) in uniform thickness and dried under the conditions of 25 degrees C/ 40% RH (To visualize, TiO₂ powder was sprinkled on the film).

Cracks can be seen as black lines.



Human Use Test of WSK In Vivo Water Sorption-Desorption Test

Hydration State of stratum corneum was evaluated by "In Vivo Water Sorption-Desorption Test" according to the method of Tagami et al.*

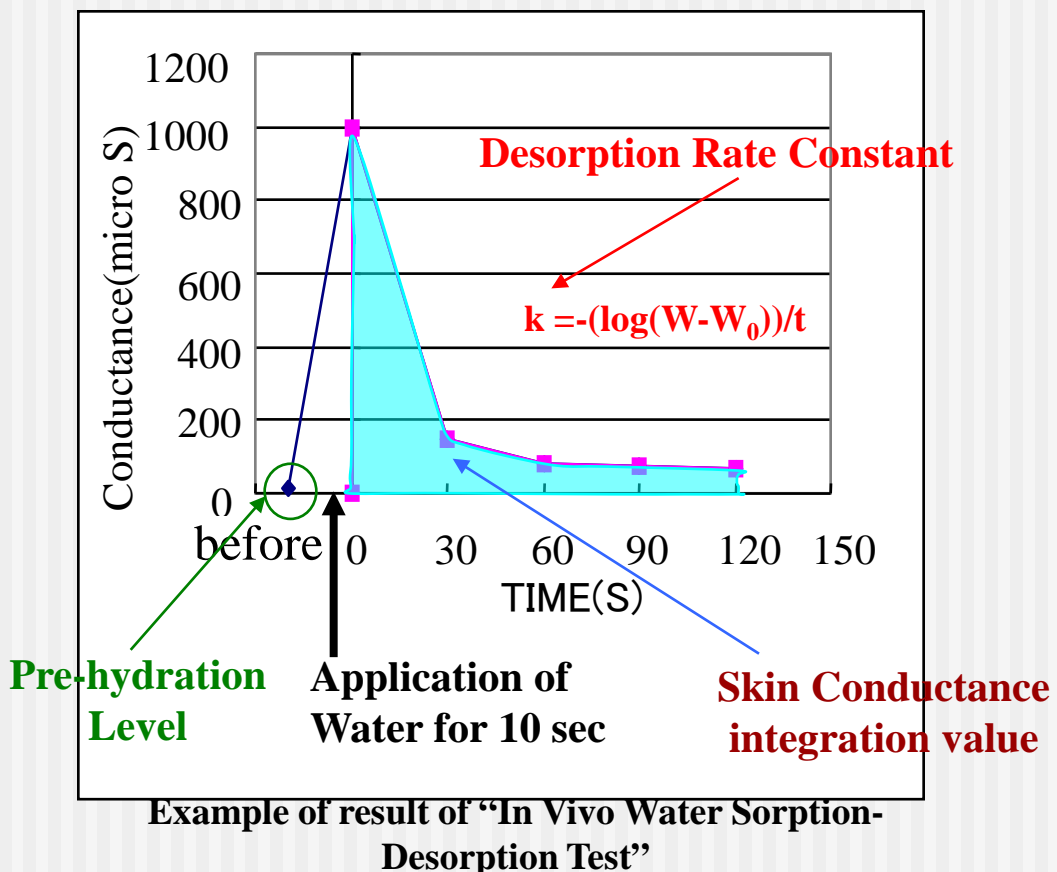
* Tagami et al., J.Investigative Dermatology,78,425(1982)

<Subjects> Forearm of healthy human adult.

<Instrument> Skicon-200 (IBS Ltd.,)

Test Procedure:

1. Test area 2cm in diameter was marked on skin with a oil-based ink to avoid missing the site in repeat measurements.
2. Forearm was washed mildly in warm water.
3. Conductance of the test area was measured by Skicon-200 more than 30 min after washing at 20°C, 40%RH.
4. At first conductance area was measured to obtain a pre-hydration level.
5. Then, a drop of distilled water was placed over the test area for 10 sec.
6. Immediately after blotting the site with a gauze, a measurement was made and repeated at an interval of 30 sec for 2 min.



Human Use Test of WSK

Functional parameters of the Test

Pre-hydration Level(Indicator of moist);

Conductance value measured just before water sorption is called “Pre-hydration Level”.

“Pre-hydration Level” is an important indicator representing a hydration state of stratum corneum.

Skin Conductance integration value (Indicator of Water Holding Capacity);

“Skin Conductance integration value” is a parameter indicating how stratum corneum can hold water.

Healthy skin has higher value of this parameter in comparison with rough or rugged skin.

Desorption Rate Constant (Indicator of Skin Barrier function);

Time course of water desorption approximates to the following exponential equation.

$$W=W_0 \cdot e^{-kt}$$

W;conductance

k; Desorption Rate Constant

t;time

The more value of k becomes small, the more skin-barrier function is improved.

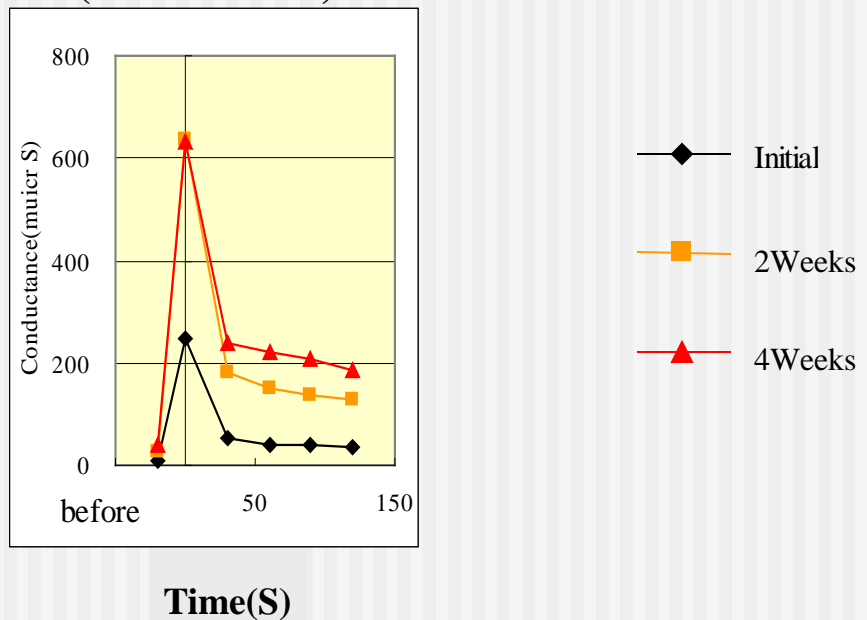
Test formulations

	Test	Reference
WSK	0.2	-
SODIUM HYALURONATE	-	0.2
PHENOXYETHANOL	0.2	0.2
METHYLPARABEN	0.1	0.1
WATER	To 100	To 100

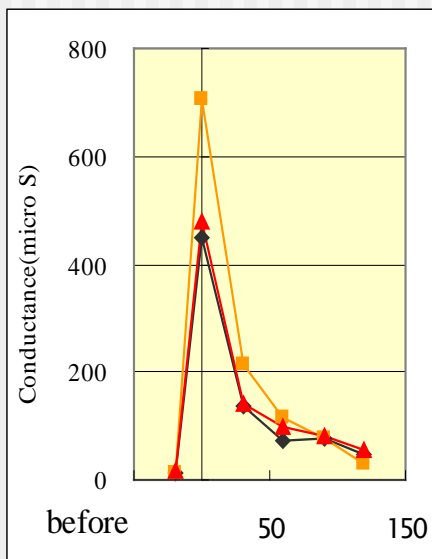
Human Use Test of WSK Improvement of Skin Hydration State (typical examples)

Each lotion was applied to the skin (forearm of healthy human adult) twice a day for a month.

(WSK Lotion)

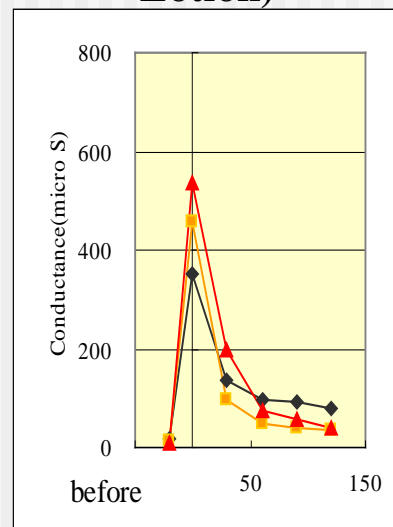


(Non Treatment)



Time(S)

(Sodium Hyaluronate Lotion)



Time(S)

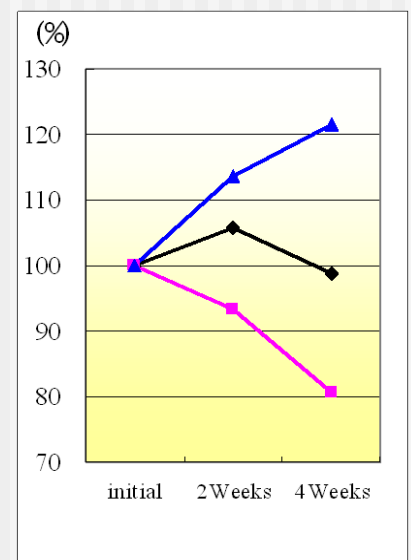
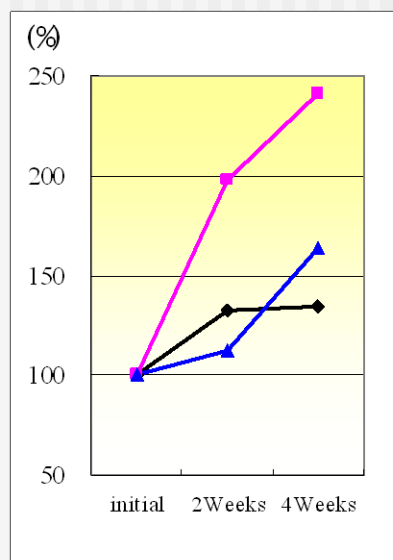
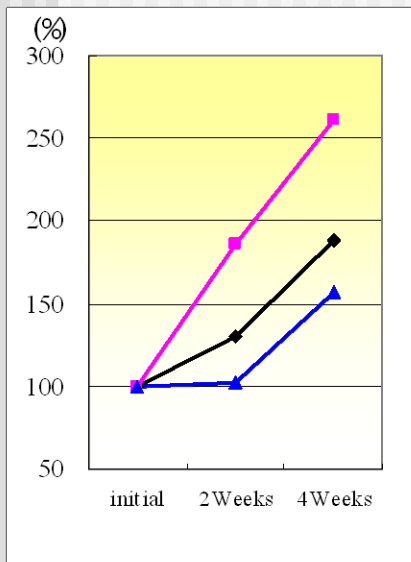
Human Use Test of WSK

Changes of the functional parameters of the Test

Pre-hydration Level
(Indicator of moist)

Skin Conductance integration value
(Indicator of Water Holding Capacity)

Desorption Rate Constant (Indicator of Skin Barrier function)



- ◆ NON TREATMENT
- WSK
- ▲ SODIUM HYALURONATE

After 2- and 4-week-application, there are obvious differences between WSK Lotion and Sodium Hyaluronate Lotion or non treatment in **Pre-hydration Level / **Skin Conductance integration value** / **Desorption Rate Constant**.**

WSK improves **Pre-hydration Level** very well. And you can get supple feeling of your skin.

WSK improves **Skin Conductance integration value** .

WSK improves **Desorption Rate Constant**.

Anti-oxidation and Anti-aging

-- WSK inhibits the action of active oxygens and prevents the aging of the skin. --

*SOD * activity of the skin cells was increased by an addition of WSK to the cultivation medium.*

<Experimental>

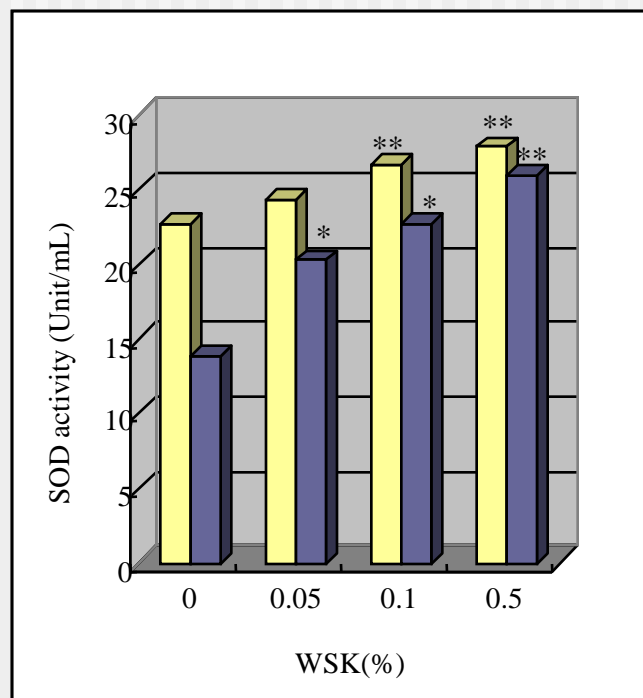
WSK was added to the culture medium cultivating keratinocyte or fibroblast isolated from rat skin. After cultivating 24 hrs. at 37 degrees C in 5 % CO₂, SOD activity was measured.

1) Keratinocyte

When added more than 0.1 % of WSK, increase of SOD activity was observed.)

2) Fibroblast

When added more than 0.05 % of WSK, increase of SOD activity was observed.



* : P < 0.05

** : P < 0.01

■ Keratinocyte
■ Fibroblast

(* SOD: SuperOxide Dismutase)

Anti-oxidation and Anti-aging

--WSK suppresses peroxidation of the cellular lipids and prevents the aging of the skin. --

WSK added to the cultivation medium suppressed lipid peroxidation in the skin cells.

<Experimental>

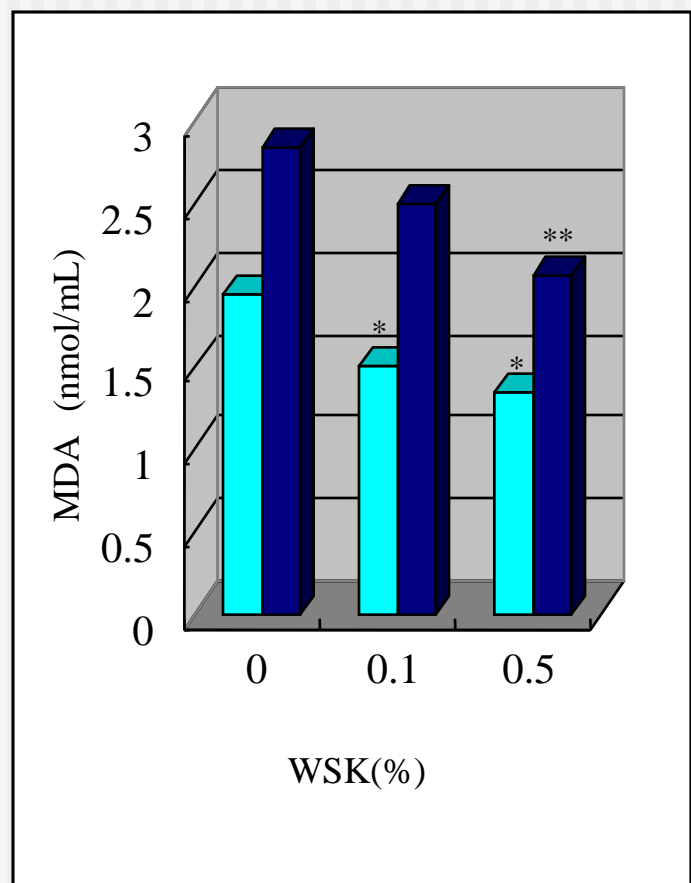
WSK was added to the culture medium cultivating keratinocyte or fibroblast isolated from rat skin. After cultivating 24 hrs. at 37 degrees C in 5 % CO₂, generated MDA* was measured as a level of peroxidized lipids by TBA** method.

1) Keratinocyte

When added more than 0.1 % of WSK, peroxidized lipids in the cell culture decreased significantly.

2) Fibroblast

When added more than 0.5 % of WSK, peroxidized lipids in the cell culture decreased significantly.



(* MDA:Malondialdehyde)

(* * TBA:Thiobarbituric acid)

* : P < 0.05

** : P < 0.01

■ Keratinocyte
■ Fibroblast

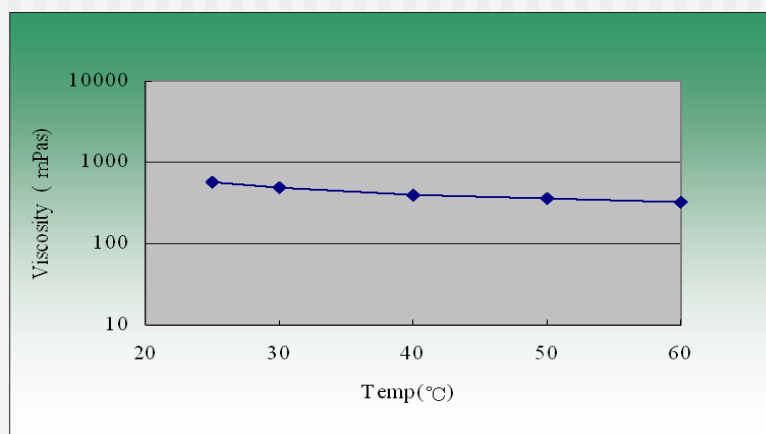
Effect of temperature and pH on the viscosity of the aqueous solution

-- Viscosity vs. Temperature --

Temperature has little effect on the viscosity of the aqueous solution of WSK.

<Experimental>

Viscosity of the 1 % aqueous soln. was measured by cone-plate type viscometer (Haake, Roto Visco RV-1). Share rate: 100(1/S)



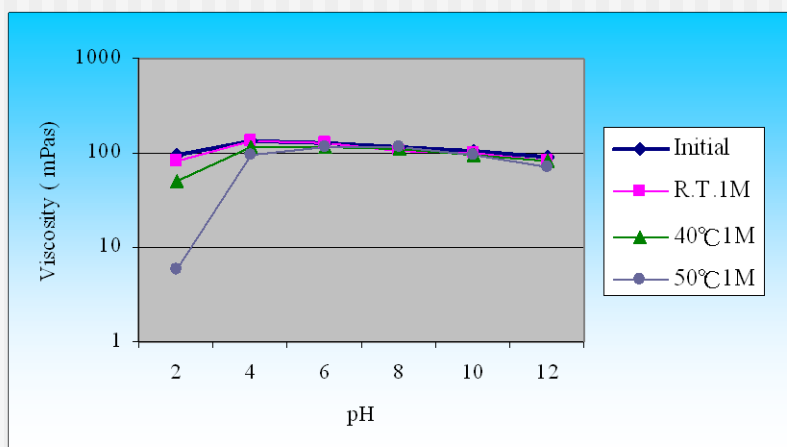
Viscosity - Temperature

-- Viscosity vs. pH --

Viscosity of the aqueous solution is stable in wide pH range (pH > 3) even at 50 degrees C for long period.

<Experimental>

Viscosity of the 0.5 % aqueous soln. adjusted at a given pH by citric acid and KOH was measured for one month. Viscometer; Haake, Roto Visco RV-1, Share rate: 100(1/S)



Viscosity - pH

Effect of salts on the viscosity of the aqueous solution

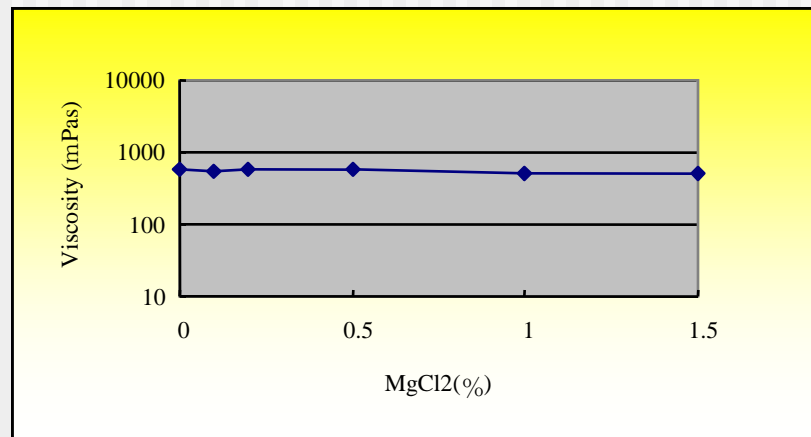
-- Salt tolerance of the viscosity --

Salts has little effect on the viscosity of the aqueous solution of WSK.

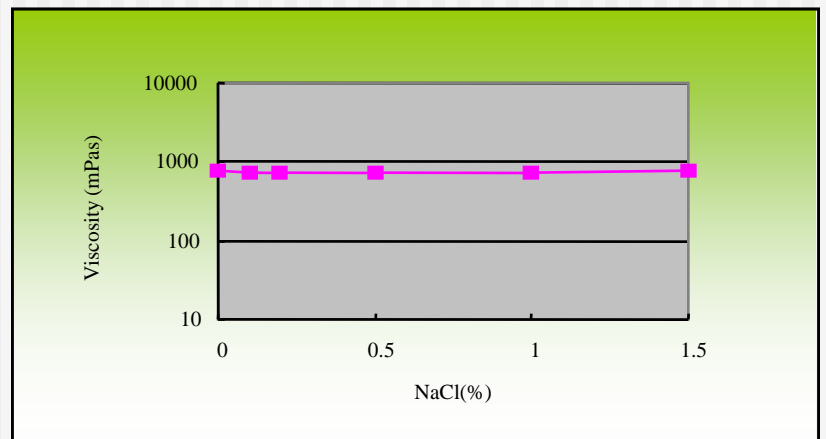
<Experimental>

Viscosity of the 1 % aqueous soln. added a given amount of salt (MgCl₂, NaCl, Zinc phenol sulfonate) was measured at 25 degrees C. Viscometer; Haake, Roto Visco RV-1, Share rate: 100(1/S)

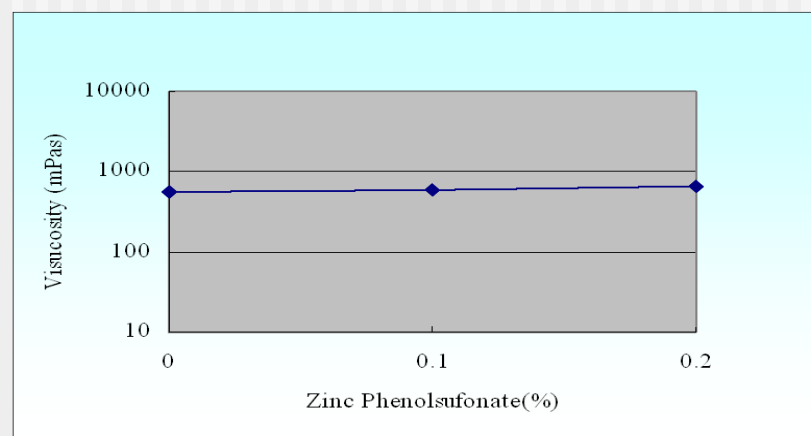
<MgCl₂>



<NaCl>



<Zn>



Sensory Evaluation of Formulations

** Moisturizing Cream **

Cream containing WSK has good spreadability and affinity, make skin supple and gives moistness.

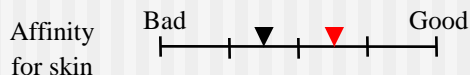
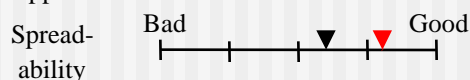
No.	Ingredients	(%)	(%)
1	WSK	0.1	-
1	SODIUM HYALURONATE	-	0.2
2	SIMMONDSIA CHINENSIS (JOJOBA) SEED OIL	3.0	3.0
3	SQUALANE	3.0	3.0
4	BEHENYL ALCOHOL	1.8	1.8
5	STEARYL ALCOHOL	1.2	1.2
6	HYDROGENATED PALM OIL	2.5	2.5
7	GLYCERYL STEARATE	1.8	1.8
8	PROPYLENE GLYCOL STEARATE SE	1.0	1.0
9	PEG-60 GLYCERYL ISOSTEARATE	1.1	1.1
10	ETHYLPARABEN	0.1	0.1
11	DIMETHICONE	1.5	1.5
12	BUTYLENE GLYCOL	6.0	6.0
13	GLYCERIN	2.0	2.0
14	PHENOXYETHANOL	0.2	0.2
15	METHYLPARABEN	0.15	0.15
16	WATER	To 100	To 100

▼ 0.1% WSK formula

▼ 0.2% Sodium Hyaluronate formula

Sensory Evaluation

< Application >



< After Application >



** Procedure **

- 1) Heat No.2~No.11 to 70°C and mix until uniform. (Part-A)
- 2) Heat No1, No.12~No16 to 70°C and mix until uniform. (Part-B)
- 3) Add Part-B to Part-A and homogenizing for 3 minutes. Cool to 40°C.

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Formulations

** Lotion **

Lotion containing WSK has good spreadability and moistness, and make skin supple and not sticky.

No.	Ingredients	(%)	(%)
1	WSK	0.2	-
1	SODIUM HYALURONATE	-	0.2
2	CITRIC ACID	0.09	0.09
3	SODIUM CITRATE	0.01	0.01
4	PHENOXYETHANOL	0.2	0.2
5	METHYLPARABEN	0.1	0.1
6	WATER	To 100	To 100

Sensory Evaluation

<Application>

Smoothness



<After Application>

Moistness



Suppleness



Sticky



** Procedure **

1) Heat half part of No.6 and No.4,5 to 70°C and mix until uniform. Cool to 40°C.(Part-A)

2) Add half part of No.6 and No1 and mix until uniform.(Part-B)

3) Add Part-B , Part-A and No.2, No.3 and mix until uniform.

** Hair Mist **

Hair Mist containing 0.02% WSK makes hair moist and gives airy feeling.

No.	Ingredients	(%)
1	WSK	0.02
2	BUTYLENE GLYCOL	1.0
3	GLYCERIN	3.0
4	SODIUM PCA	0.3
5	ALCOHOL	5.0
6	SODIUM CITRATE	0.1
7	METHYLPARABEN	0.1
8	WATER	To 100

** Procedure **

Heat No.1~8 at 80°C and stir until uniform.Cool to 40°C.

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Specification

	Specification (interim)
Appearance	White to pale yellow powder. Slight characteristic odor
Identification: Saccharide	Deep red
Identification: Glucuronic Acid	Amaranth(or peachblow)
Loss on Drying	< 10 (%)
Residue on Ignition	< 10 (%)
Transparency	> 90 (%)
PH	5.0~7.0
Heavy Metals	< 20 (ppm)
Arsenic	< 2 (ppm)
Quantitative: Content of Total Saccharide	> 80 (%)
Quantitative: Content of Glucuronic Acid	10~30 (%)

Aerobic plate count (/g)	< 200
Total combined molds and yeasts count (/g)	< 100
Coliform bacteria	negative
Coagulase positive Staphylococci	negative
Pseudomonas aeruginosa	negative

Safety Profile

Acute Eye Irritation	NON IRRITANT
14-Days Repeat Application Dermal Irritation	NON IRRITANT & NON SENSITIZER
Acute oral toxicity/pathogenicity	NON TOXICANT

Storage

Store in sealed container in cool, dry and dark conditions.

R & D

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