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A Study on the Hydrolyzed Ginseng Saponin-Quaternary and its Application in Cosmetics

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Abstract□A new quaternary ammonium compound, hydrolyzed ginseng-saponin quaternary (HGSQ), from Korean ginseng saponin and 2,3-epoxypropyltrimethylammonium chloride has been developed as a conditioning agent in cosmetics. This structure has a hydrophobic group from the aglycone of ginseng saponin which is biologically active and considered to be the most important component of the Korean ginseng. Its properties: surface tension, critical micelle concentration (CMC), eye irritation, sorption onto hair, force reduction (%) and moisture retention effect were studied. Its cationic character allows the molecule to be more substantive than ginseng saponin. HGSQ had good physical properties and was safe enough as a cosmetic raw material. Also half-head tests of HGSQ-containing shampoo were carried out to compare the conditioning effects in shampoos. HGSQ was an excellent conditioning agent in shampoo.

Keywords□ginseng, hydrolyzed ginseng-saponin quaternary, conditioning agent, critical micelle concentration, eye irritation, sorption, force reduction (%), moisture retention effect.

Introduction

Ginseng saponin has good effects on skin and hair[1]. But it has poor adherent property in rinse-off skin and hair care products. Skin and hair have an overall negative charge at normal condition, they are ideal for interaction with cationic materials. Cationics are substantive because of the interaction of their positive charge with the substrate's negative charge. The cationic material improves the texture of dry hair and softens it. It neutralizes the apparent anionic charge of hair and therefore eliminates static flyaway effect[2]. Its positive charge on the cationic helps the hair hold onto water and increase the hydrophilic nature of hair. The key to its effects is the substantivity to hair[3]. It was necessary for us to find out the more effective method of using the precious ginseng saponin in cosmetics. In this study, we have improved the substantivity of ginseng saponin to hair by introducing the cationic group to the aglycone of ginseng saponin.

Experiments

Chemicals and Instruments

NMR spectra were recorded at 60 MHz with tetramethylsilane (TMS) as an internal standard using Varian EM 360L spectrometer. Melting points were measured by Yamato Mp-21 melting point apparatus and were uncorrected, using open capillaries. Thin layer chromatograms (TLC) were run on silica gel 60F (Merck) and the spots were visualized with iodine vapor or Dragendroff reagent. Rheo Meter, Fudo model 200-2J, was used for the measurement of the force reduction(%). Surface tensions were measured by Fisher autotensiometer (model 215). A spectrophotometer, Ceil Series 5000, was used for the measurement of uv-visible absorption spectra. 2,3-epoxypropyltrimethylammonium chloride was obtained from Keon Chang Chemical Co. and used without purification. Ginseng saponins were prepared from Korean ginseng by Pacific Chemical Co., Ltd. All other

reagents were of reagent grade commercially available.

Hydrolysis of ginseng saponin

Crude ginseng saponin (30g) was partitioned in n-BuOH-H_2O (1:1) mixture as usual, and n-BuOH layer was evaporated in vacuum to give a residue, which was dissolved in MeOH-H_2O solution. Methanol soluble portion was then treated with a large amount of ether to produce precipitate, which was collected and dissolved again in BuOH-H_2O mixture. BuOH layer was dried under vacuum. 25g of ginseng saponin was dissolved in 200 m*l* of distilled water at 40°C and 18g of conc. sulfuric acid was dropped into reaction vessel with efficient stirring and standard for 1 hr at 55°C. The resulting reaction mixture was filtered, washed with water five times and dissolved in chloroform. The organic layer was washed with 2% sodium bicarbonate and with water, and removed the chloroform on a rotary evaporator to give yellowish solid. The solid was recrystallized from acetone-water, affording 6.1g of slightly yellow solid, mp 168-171°C. The degree of reaction was checked by TLC on silica gel, Rf: 0.18, 0.26, 0.45 (major), 0.55 solvent: Benzene-Acetone(4:1).

Preparation of hydrolyzed ginseng-saponin quaternary

6g of HGS (hydrolyzed ginseng-saponin) was suspended in 300 m*l* of isopropyl alcohol. 8.6g of 2,3-epoxypropyltrimethylammonium chloride (70% aq-sol'n) was solwly added to the solution of HGS followed by an equimolar amount of sodium hydroxide. The reaction was continuted for 8 hr at 55°C. After the reaction was completed, the reaction solution was neutralized with 0.4 m*l* of acetic acid for 20 min, and then concentrated to syrup on a rotary evaporator. The crude product was applied to a column of Sephadex LH-20 and eluted with methanol. Fractions were collected and every fraction was checked by TLC on silica gel (solvent; chloroform/methanol/water = 13:7:2, lower phase) using Dragendorff reagent [4]. After pooling the main product fractions, methanol was evaporated under reduced pressure. The yield was 6.5g of pale yellow solid, mp 240-242°C. TLC with chloroform/methanol/water(13:7:2, lower phase) on silica gel gave three spots, Rf: 0.17 (major), 0.59, 0.85. Nitrogen analysis of HGSQ indicated that contains 2.4% nitrogen. Average molecular weight of HGSQ determined by the freezing point depression and nitrogen analysis was about 557.

Critical Micelle Concentration(CMC)

CMC's of HGSQ and ginseng saponin were determined by measuring the surface tension at 10^{-3} to 2% (w/w) concentrations at 20 ± 2°C.

Eye irritation

0.5% active solutions of HGSQ and ginseng saponin were prepared. Six normal healthy albino rabbits were used in this experiment. The scores were recorded according to the Draize scoring ocular lesions[5].

Determination of sorption amounts

The hair tresses (1g and 4cm long) were prepared from virgin black hairs (25 cm long) of female Koreans aged 20-30 and shampooed with 1% Triton X-100 aqueous solution, rinsed with distilled water and dried. Half of the tresses were bleached by ammonium hydroxide (0.5%) and hydrogen peroxide (6%) at 20±2°C. Each hair tress was put into the beaker of 50 m*l* mixed bleaching solution for given times. After bleaching, the tresses were shampooed twice with 1% Triton X-100 solution and rinsed twice with distilled water. All the tresses were conditioned in a humidity controlled chamber to 60± 4% RH at 20±2°C.

1 g of hair tress was immersed in 100 m*l* Erlenmeyer flask with 50 m*l* of sample solutions of HGSQ and ginseng saponin at 20±2°C for given concentrations and times in a shaker.

The sorbed HGSQ was extracted with 30 m*l* of 80% methanol solution three times from the treated hair tresses respectively. The extracted solution from hair tress was evaporated in vacuum. The residue was dissolved with small amount of water and made up to about 40 m*l* with water and about 1

m*l* of 0.1% (w/w) solution of picric acid in 0.002 M sodium hydroxide added. 20 m*l* of distilled chloroform was added to the whole solution, which was shaken for 5 min. The organic layer was then removed and centrifuged at 3500 rpm for 5 min. The resulting picric acid-quaternary ammonium compound complex in chloroform was determined at 360 nm[6-8].

The sorbed ginseng saponin was extracted with 30 m*l* of 80% methanol solution three times from the hair tress. The solution was evaporated in a rotary evaporator. The residue was dissolved into 2 m*l* of 80% methanol solution. Then the solution was eluted with CH_3CN: H_2O (28.5:71.5) on μ-Bondapak C18 (3.9 mm × 30 cm) and the absorbance of the ginseng saponin was determined at a wavelength of 203 nm (flow rate: 3.0 m*l*/min)[9].

Force reduction (%)

Hairs were bleached and rinsed as in sorption test. The hairs with diameter between 80 and 90 μm were selected microscopically. Both ends of hair were protected from damages by a Scotch filament tape.

First, the bleached hairs (100 pieces) were conditioned at 60±4% RH and 20±2°C for 10 hr, and the force for 20% extension was measured. The hairs were relaxed overnight (15 hr) in the water and treated with 50 m*l* solutions of test materials at given concentrations (0.05 to 5%), immersion times (10 to 180 min and 15 hr) and rinsed twice with 50 m*l* distilled water and conditioned again. Next, the force for 20% extension of the treated hairs was measured again. Rheo Meter (model 200-J Fudo Kogyo Co., Japan) with multipen recorder was used. Under the conditions of initial hair length 50 mm, the rate of extension was 5 cm/min and full scale was 200 gf.

Moisture retention effect

2g of the virgin and bleached hair tresses were prepared and conditioned at 20% RH. After that, the equilibrium weight of the tresses was measured. Then the tresses were treated with 50 m*l* solutions of 0.5% test materials at 20±2°C for 15 hr, rinsed with 50 m*l* distilled water twice and finally conditioned at given relative humidities (20 to 95%) for 10 hr at 20±2°C. Measurements of the weight of the hair tresses at given conditions were carried out on an electrobalance to ±0.1 mg accuracy in humidity controlled room at 20±2°C. Under the same conditions moisture retention effects of HGSO itself were also compared with the commercial standards and propylene glycol.

Evaluation of HGSQ-containing shampoo

For this study, we wanted a clear simple formula with as few ingredients as possible so as not to interfere with the conditioning properties of the test materials during their evaluation.

SHAMPOO FORMULATIONS

INGREDIENTS	A	B	C
SLES (30%)	40.0	40.0	40.0
COCOAMPHOCARBOXY-PROPIONATE (70%)	10.0	10.0	10.0
LAUROYL MYRISTOYL DEA	5.0	5.0	5.0
HGSQ	2.0	—	—
GINSENG SAPONIN	—	2.0	—
PROPYLENE GLYCOL	3.0	3.0	3.0
METHYL PARABEN	0.2	0.2	0.2
CITRIC ACID to pH 6.0	q.s.	q.s.	q.s.
PERFUME	q.s.	q.s.	q.s.
DEIONIZED WATER to	100.0	100.0	100.0

Above three shampoos were prepared for the half-head test[10] involving 20 subjects which were divided into 2 groups by 10 subjects. All the subjects were females aged 15-40. Half of them had permed hair. The test was carried out in a professional beauty salon. The HGSQ-containing shampoo and other shampoos were tested for each group. For assessment, the subject and the operator choose the better one of the two shampoos tested on the subject by evaluating the left and the right part of the subject's hair. The effects of the shampoos were evaluated in regard to combability, feel of wet and dry hair as well as sheen and antistatic effects of dry hair.

The hair was parted in the middle and combed

out. One side was wetted, then washed with a measured amount (ca. 5-10g) of shampoo A according to the hair volume. The other side was washed with the same amount of the counter shampoo. At this point, foam quality and quantity were observed. The procedure was repeated again. After the final rinse, wet combability and feel were evaluated by hand with a comb by the subject and the operator. The subject was placed under the dryer until the hair was thoroughly dry. After completion of the drying, the hair was evaluated for combability, feel, sheen and antistatic effect. The final decision of the effects of dry hair was made after checking both states just after drying and 24 hr later. Antistatic effect was evaluated by checking the flyaway of hair just after combing by hand. For each effect 1 point was given for the chosen shampoo and 0 for the other by each subject. Also the operator gave the points as above again for the 2 shampoos used for evaluation.

Results and Discussions

Hydrolysis of ginseng saponin

Ginseng saponin was hydrolyzed with sulfuric acid and washed with water several times. The resulting solid was fractionated with chloroform-water. The chloroform extract showed spots on TLC. In the NMR spectrum, the sugar peaks of ginseng saponin disappeared after hydrolysis. The peaks of δ4.4 and 4.8 in ginseng saponin nearly disappeared in nmr spectrum of hydrolyzed ginseng-saponin (HGS).

Preparation of hydrolyzed ginseng-saponin quaternary (HGSQ)

The quaternization of HGS was achieved by etherifying HGS with 2,3-epoxypropyltrimethylammonium chloride and an equimolar amounts of sodium hydroxide. 2,3-epoxypropyltrimethylammonium chloride is good electrophile owing to its strong inductive effect of neighboring quaternary ammonium salt. So the hydroxy group of HGS easily react with 2,3-epoxypropyltrimethylammonium chloride by nucleosubstitution reaction.

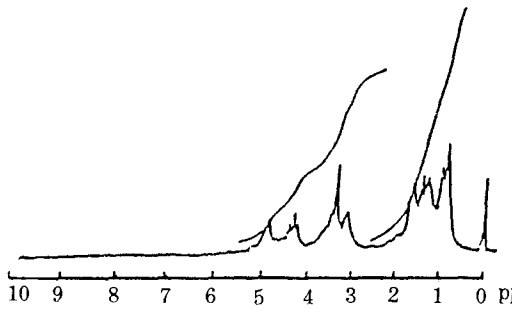

Fig. 1. The nmr spectrum of ginseng saponin, Solvent, DMSO-d_6.

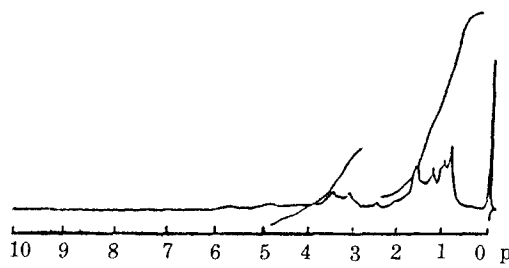

Fig. 2. The nmr spectrum of hydrolyzed ginseng-saponin, Solvent, DMSO-d_6.

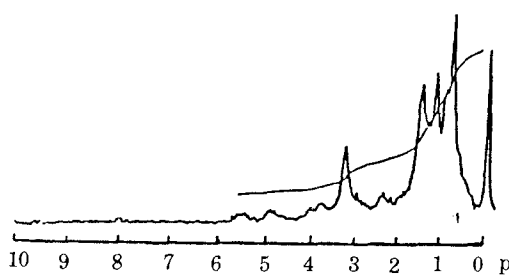

Fig. 3. The nmr spectrum of HGSQ, Solvent, DMSO-d_6.

The structure of HGSQ was confirmed by nmr spectrum. As shown in Fig. 3, the single peak at δ3.1 is indicative of quaternary ammonium group of HGSQ. Average molecular weight of HGSQ was determined by the freezing point depression and nitrogen analysis.

Critical micelle concentration (CMC)

The test results are shown in Fig. 4. The CMC value of HGSQ by surface tension at 20 ± 2 °C was 0.01% (w/w). Ginseng saponin had the CMC value of 1.0% (w/w), which is much larger than that of

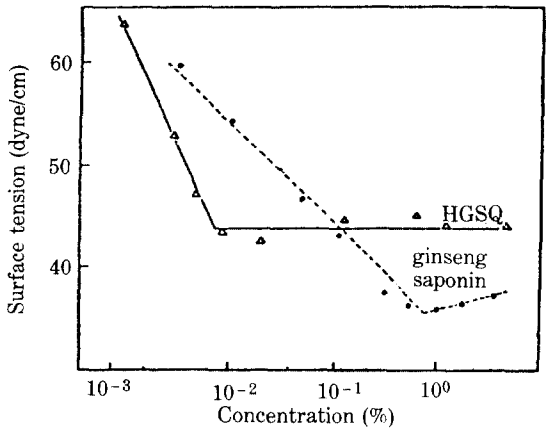

Fig. 4. The effect of concentration of surface tension (temp. 20±1°C).

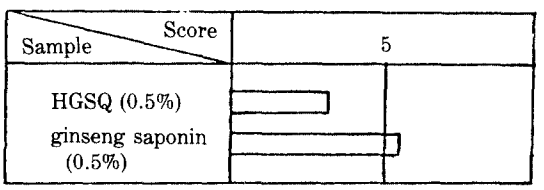

Fig. 5. Draize eye irritation scores.

HGSQ and almost the same value by Kim[11]. By these facts, we found that HGSQ had a reasonable surface activity. Its small CMC value maybe comes from the particular aglycone of ginseng saponin.

Eye irritation

As shown in Fig. 5, HGSQ was proved to be less irritant than ginseng saponin. HGSQ, even though it is a quaternized compound from ginseng saponin, is less irritant than ginseng saponin itself. It is generally considered that cationic surfactants are more irritant than nonionic and anionic surfactants. Using the Chamber Scarrification Test for assessing irritancy, Frosch and Kligman found that the general decreasing order of irritancy is as follows: cationics>anionics>nonionics[12]. But it is very interesting that HGSQ showed less irritancy than ginseng saponin which is thought to be extremely nontoxic[13]. Maybe it comes from the characteristic property of ginseng saponin aglycone.

Sorption onto hair

In Fig. 6, the sorption amount of test materials

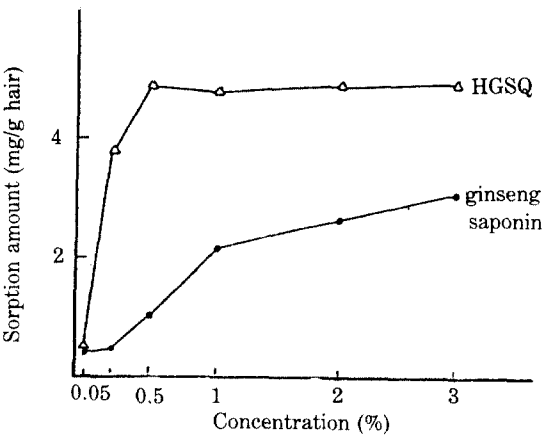

Fig. 6. The effect of concentration on sorption amount of HGSQ and ginseng saponin into virgin hair (immersion time 3 hr; temp. 20±2°C; pH 6.0±0.1).

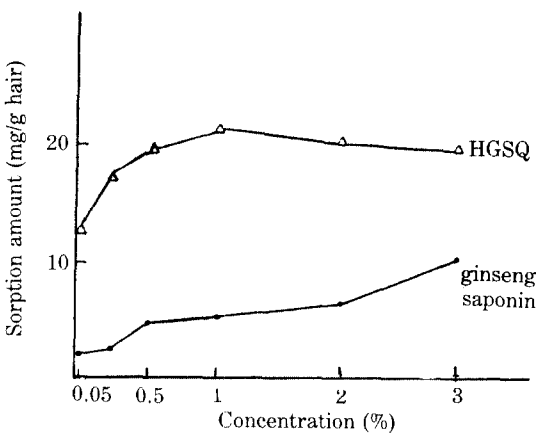

Fig. 7. The effect of concentration on sorption amount of HGSQ and ginseng saponin into bleached hair (immersion time 3 hr; temp. 20±2°C; pH 6.0±0.1).

on virgin hair are shown. At low concentration of 0.05 to 0.5%, sorbed amount of HGSQ was much more than ginseng saponin. HGSQ was not more sorbed at the concentrations above 1.0%. But in case of saponin, the sorbed amount was increased to the concentration of 3.0% (w/w). HGSQ showed much higher sorption amount onto hair than ginseng saponin in this condition.

The sorbed amount onto bleached hair was much higher than onto virgin hair as expected (Fig. 7). HGSQ showed much higher sorption amount at 0.05% than ginseng saponin. At higher concentra-

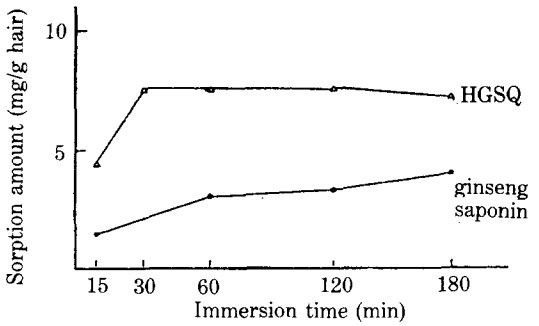

Fig. 8. The effect of immersion time on sorption amount into virgin hair (conc'n 5%; temp. 20±2°C; pH 6.0±0.1; RH. 60±4%).

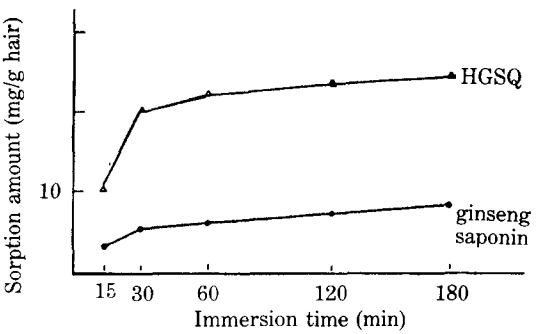

Fig. 9. The effect of immersion time on sorption amount into bleached hair (conc'n 5%; temp 20±2°C; pH 6.0±0.1; RH. 60±4%).

tion, its sorption amounts was slightly decreased by increasing the concentration up to 3.0% (w/w).

The sorption amounts onto virgin hair and bleached hair at given immersion times (15 to 180 min) are shown in Fig. 8 and 9. HGSQ showed higher sorption amounts than ginseng saponin. These results explain the higher substantivity of HGSQ than ginseng saponin in this condition.

Force reduction (%)

In Fig. 10, force reductions(%) for 20% extention of the hairs at given concentrations are shown. HGSQ showed much lower value of force reduction (%). Force reduction (%) decreased gradually by the increase of the concentration of HGSQ. Force reductions (%) at given immersion times are also shown in Fig. 12. HGSQ also showed much lower value than ginseng saponin, but its value was not decreased after 60 min immersion. But in case of

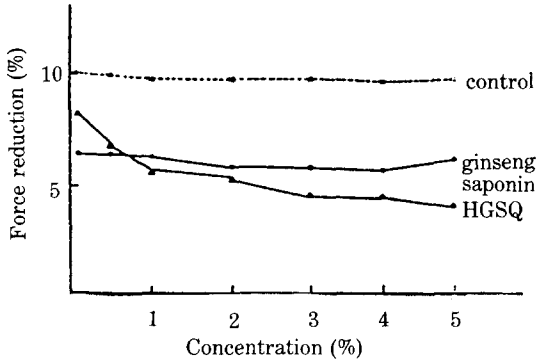

Fig. 10. The effect of concentration on force reduction (%) of bleached hair (bleaching time 1 hr; immersion time 15 hr; temp. 20±2°C; RH. 60±4%).

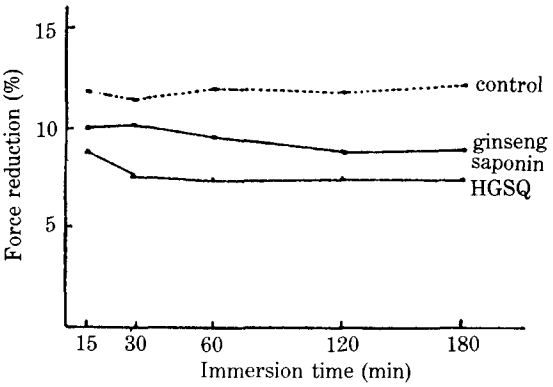

Fig. 11. The effect of immersion time on force reduction (%) of bleached hair (bleaching time 1 hr; conc'n 5%; temp. 20±2°C; RH. 60±4%).

ginseng saponin, the values were decreased until to 120 min.

Force reductions (%) at given bleaching times are shown in Fig. 11, which were rapidly increased according to the bleaching times. HGSQ showed lower values of force reduction (%), which means HGSQ modifies the tensile strength of damaged hair more effectively than ginseng saponin. This is maybe possible by the much sorption of HGSQ into hair followed by the binding of HGSQ with amino acids, cysteic acid, in the hair cortex.

The % reduction in force required to extend a fiber 20% after treatment was calculated using the following equation,

$$\text{Force reduction (\%)} = \frac{F_1 - F_0}{F_0} \times 100$$

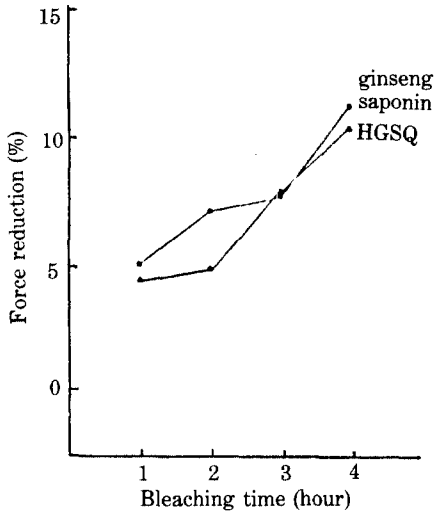

Fig. 12. The effect of bleaching time on force reduction (%) of bleached hair (immersion time 15 hr; conc'n 5%; temp. 20±2°C; RH 60±4%).

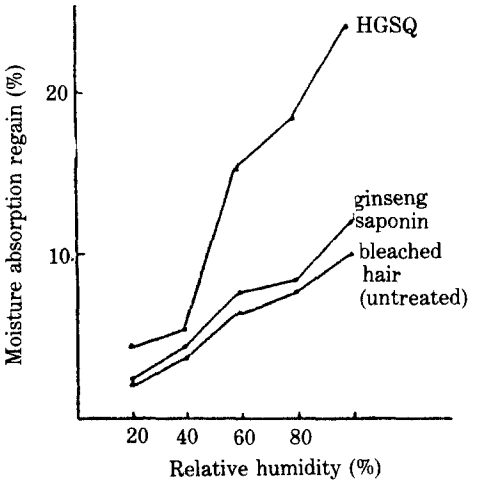

Fig. 14. The moisture absorption regain isotherms of bleached hair treated with HGSQ and ginseng saponin (immersion time 15 hr; conc'n 0.5%; temp. 20±2°C).

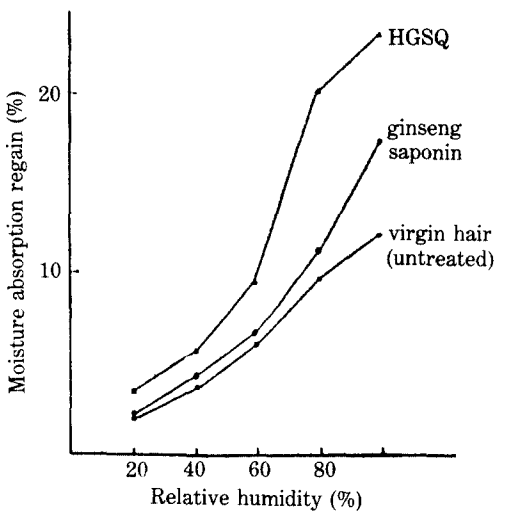

Fig. 13. The moisture absorption regain isotherms of virgin hair treated with HGSQ and ginseng saponin (immersion time 15 hr; conc'n 0.5% temp. 20±2°C).

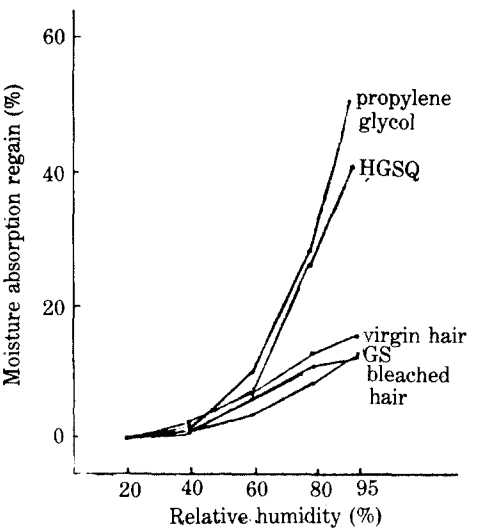

Fig. 15. The moisture absorption regain isotherms of propylene glycol, HGSQ, ginseng saponin, virgin hair and bleached hair (temp. 20±2°C).

where F_0 is the initial force required to extend a fiber 20% and F_1 is the force required to reextend the same fiber to 20% after treatment[14].

Moisture retention effect

Moisture absorption regain (%) isotherms for the virgin hair treated with HGSQ and ginseng saponin are shown in Fig. 13. It showed that HGSQ treated hair had much higher moisture absorption regain (%) than others, especially at high RH. Untreated virgin hair showed lowest value. The results of the bleached hair are shown in Fig. 14. Bleached hair treated with HGSQ showed the highest moisture absorption regain (%) especially above 40% RH. In Fig. 15, the test materials' isotherms are shown. As

Table 1. Half-head test results by the subjects and the operator

Group		1		2	
Part of hair		Left	Right	Left	Right
Shmpoo		A	B	C	A
Wet hair	Feel	12	8	8	12
	Combability	11	9	9	11
Dry hair	Feel	10	10	7	13
	Combability	12	8	7	13
	Sheen	11	9	9	11
	Antistatic effect	12	8	10	10
Wet hair total points		23	17	17	23
Dry hair total points		45	35	33	47
Total points		68	52	50	70
Preference (%)		57	43	42	58

expected, virgin hair showed higher moisture absorption regain (%) than bleached hair. HGSQ showed higher moisture absorption regain (%) than ginseng sapoinin, virgin hair and bleached hair. HGSQ showed very similar value to that of propylene glycol at up to 80% RH but lower value than propylene glycol at 95% RH. By these results we found that HGSQ itself had good moisture retetion effect and made hair retain much moisture.

$$\text{Moisture absorption regain}(\%) = \frac{W_1 - W_0}{W_0} \times 100$$

where W_0 is the weight at initial condition ($20 \pm 4\%$ RH, 20 ± 2 °C) and W_1 is the weight at given conditions.

Evaluation of HGSQ-containing shampoo

The results of half-head test are shown in Table 1. The numbers mean the chosen times by the subjects and the operators as a better shampoo. As shown in Table 1, HGSO-containing shampoo A is an attractive product. In washing, it gave rich and dense foam to the hair. All the tested shampoos except shampoo C gave wet hair smooth feels. Shampoo A made hair much easier to comb than shampoo B and C. Shampoo A gave excellent properties in soft feel, combability, sheen and anti-static effect when dry. These maybe come from the unique properties of HGSQ: high moisture retention effect, high sorption capacity onto hair due to its cationic character.

Acknowledgements

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