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Hydagen® Aquaporin

Booster of cellular water channels

Global Except US

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SUMMARY FILE

Hydagen® Aquaporin	
Origin - Description	Glyceryl Glucoside (and) Glycerin
Regulatory data	
INCI	Glyceryl Glucoside (and) Glycerin
China	Each component is listed in Inventory of Existing Cosmetic Ingredient in China (IECIC 2021)
CAS#	100402-60-6 / 56-81-5
EINECS#	309-496-6, 200-289-5
Appearance	Clear to opalescent viscous liquid
Preservative	None
Halal	Halal certified
Natural labels	Raw material conform to COSMOS standard of natural and Organic Cosmetics
Naturalness content (ISO 16128)	100% from natural origin
Cosmetic use	
Properties	Helps the transport of moisture in the skin Helps to stimulate the aquaporin water channels between the skin cells Helps to protect aquaporin water channels against blue light (additional data 2022) Helps to strengthen the skin barrier function Instant and 24h hydration Boosts the hydration capacity of glycerin Skin hydration and smoothness benefits demonstrated also in rinse off formula (additional data 2022)
Applications	Face care, body care, scalp care, men care, baby care, lip care, cleansing, shower Particularly suitable for dry and damaged skin and scalp
Formulation data	
Concentration of use	1.5 - 5%
Solubility	Soluble in water
Incorporation method	Hydagen Aquaporin is compatible with all kinds of formulation. It can be incorporated during the process up to 80°C, or at room temperature for cold processing
Optimal pH	3 - 11
Patent family	EP2099424B1, CN101453979B1

AQUAPORINS: CELLULAR WATER PORES

Discovery of the cellular water pores, the aquaporins, awarded by the Nobel Prize in Chemistry

The first water channel was identified in 1988 by Peter Agre [Denker *et al*, 1988], who alongside Roderick MacKinnon won the Nobel Prize in Chemistry in 2003 for this discovery [NobelPrize.org]. He has clarified how water is transported out of and into the cells.

The cell membranes are far from being an impervious shell, but are perforated by various channels, to ensure the passage of small molecules and ions for communication between cells. Selectivity is a central property of many of these channels, which permits one specific ion or molecule to pass through each of them.

Water channels are found in all organisms, from bacteria to humans. They allow the cell to regulate its volume and internal osmotic pressure.

The existence of channels mediating the flow of water and small solutes through the membrane of individual cells was postulated as early as in the 1850s. In the late 1950s, it was discovered that water could be rapidly transported in and out of cells through pores that admit water molecules only. From 1988 to the early 1990s, Peter Agre and co-workers isolated a new 28 kDa membrane protein and demonstrated that the expression of this protein into cell was necessary for making the cell absorb water and swell [Preston *et al*, 1992]. Also, they found that « artificial cells » (liposomes) became permeable to water if this protein was introduced in their membranes. Agre named the protein aquaporin (AQP), «water pore».

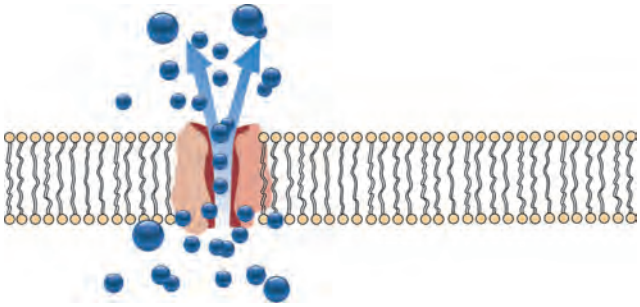


Figure 1 - Transport of water through the aquaporin (AQP).

The selectivity of the aquaporin is conferred by electrostatic and steric factors [Hub *et al*, 2009]. Through this selective narrow channel located on cell membranes (Figure 1), the water molecules worm their way through by orienting themselves in the local electrical field formed by the atoms of the channel wall. Due to the positive charge at the center of the channel, positively charged ions such as H_3O^+, are deflected. This prevents proton leakage through the channel.

Since this discovery of the first AQP by Peter Agre, at least 13 AQPs (AQP0-AQP12) have been identified to date in humans, as reviewed by several authors [Verkman, 2011; Day *et al*, 2014]. These AQP channels share a common architecture, and function as selective pores allowing water, but also for some of them, of other small solutes, in particular of glycerol. Aquaporins are thus divided into 2 major groups, those strictly selective for water -orthodox aquaporins (AQP0, 1, 2, 4, 5)- and those that, besides water, are also permeable to glycerol -aquaglyceroporins (AQP3, 7, 9, 10) (Figure 2). The other identified AQPs are to date less understood.

AQP-expressing cells generally contain several thousand, or more, AQPs per µm² of membrane, as compared with ten or fewer ion channels per µm² of membrane [Verkman, 2011].

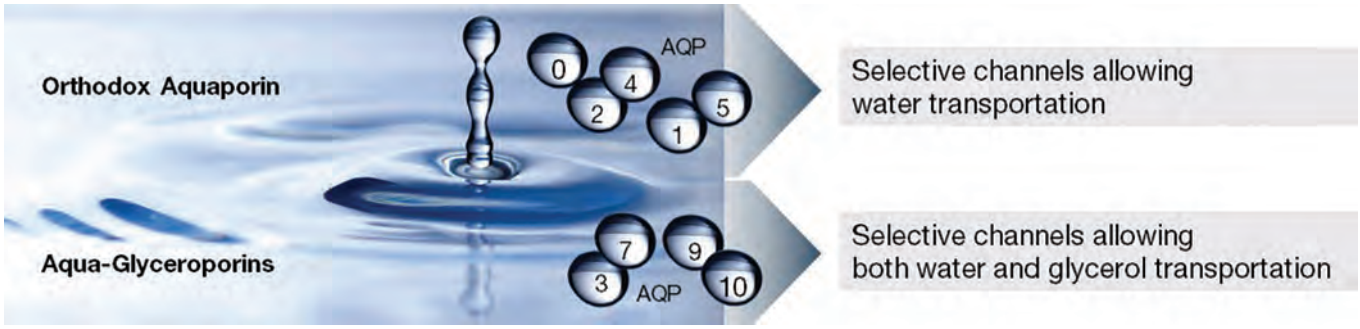


Figure 2 - Major subgroups of aquaporins.

Aquaporin-3 in the skin

Aquaporin-3 (AQP3) is an aquaglyceroporin expressed in epithelial tissues exposed to water loss conditions and is the predominant aquaporin in the epidermis [Day *et al*, 2014]. It is expressed and located mainly in the *stratum basale*, with a decreasing expression towards the *stratum granulosum*. Even if previously reported to be absent in stratum corneum (SC) [Sougrat *et al*, 2002], Jungersted and co-workers have shown the presence of AQP3 in human SC [Jungersted *et al*, 2013].

The functions of AQP3 in skin have been reported mainly from studies on AQP3-deficient mice. AQP3-null mice display impaired barrier function and reduced *stratum corneum* hydration and elasticity, which cannot be corrected by skin occlusion or exposure to a highly humide environment [Ma *et al*, 2002]. Analysis of these mice revealed a reduced glycerol content in the SC and the epidermis, whereas glycerol levels in the epidermis and the serum were normal [Hara *et al*, 2002], impaired cellular glycerol metabolism and biosynthesis, impaired lipid biosynthesis with reduced ATP content [Verkman, 2011]. Topical administration of glycerol has shown to correct many defective skin functions in AQP3-null mice [Hara & Verkman, 2003]. It has also been shown that increased AQP3 results in the retention of epidermal water [Mirza *et al*, 2008]. In addition, AQP3 expression is related to the expression of other epidermal proteins involved in water maintenance, such as filaggrin [Gasser *et al*, 2004]. These results suggest that there is a key role of glycerol transport depending on AQP3 level in epidermal hydration, elasticity and barrier function.

A study on human skin has shown that AQP3 expression gradually decreases with advancing age in sun-protected and sun-exposed skin [Seleit *et al*, 2015]. As aged skin is characterized by decreased hydration, delayed repair, increased fragility and reduced elasticity, these changes may be explained at least in part by AQP3 deficiency. UVB irradiation was also reported to reduce AQP3 expression in cultured keratinocytes [Shin *et al*, 2017]. In addition, AQP3 is believed to be important in wound healing by enhancing epidermal cell migration and proliferation [Hara-Chikuma & Verkman, 2008].

Boosting skin hydration by stimulating biosynthesis of aquaporin-3

The skin is keys in water homeostasis and provides a barrier function against excessive water loss. Skin moisturization depends on complex natural moisturizing factor located in corneocytes, content of water-holding macromolecules such as hyaluronic acid, but also on regulation of water and glycerol transport [Bonté, 2011]. This water and glycerol content are essential for normal function of the skin, and this is largely under the control of the aquaglyceroporin AQP3. AQP3 maintains glycerol in the skin to hold moisture and the levels of cellular glycerol for cell energy and metabolic needs [Verkman, 2011].

AQP3 appears to be a key protein target in epidermal homeostasis (hydration, elasticity, barrier recovery, wound healing, cellular energy and metabolism) and to improve the skin hydration state and barrier function of dry skin or scalp.

HYDAGEN AQUAPORIN: DESCRIPTION OF PRODUCT

Hydagen Aquaporin is Glyceryl Glucoside (GG), i.e. a glycerol and a D-glucose linked by a glycosidic bond (figure 3), in solution in Glycerin. It is obtained by catalytic acetalisation of these substrates, both from vegetal origin. It combines the protective effects of these two chemical classes of natural compounds, a sugar moiety and a polyol unit.

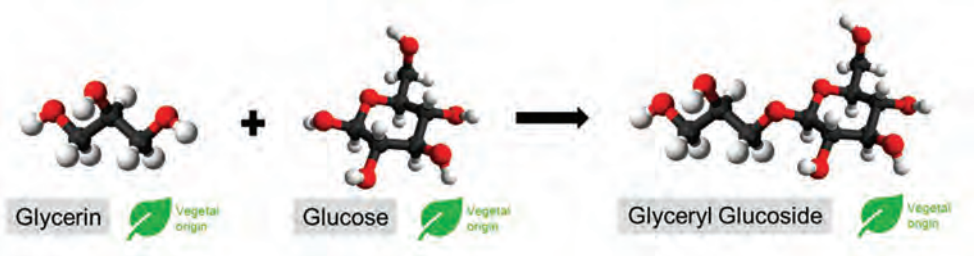


Figure 3 - Glyceryl Glucoside synthesis from Glycerin and Glucose.

INCI Name: Glyceryl Glucoside

Synonyms: Gluco-Glycerol, glucosylglycerol, ether of D-Glucose with glycerol

CAS: 100402-60-6

Glyceryl Glucoside (GG): a naturally occurring heteroside

GG is a major compatible solute characteristic from marine cyanobacteria (blue-green algae), found in about 40 different strains [Hagemann & Pade, 2015]. Under saline stress conditions, these microorganisms accumulate GG to counteract desiccation and osmotic stress. It was also found in heterotrophic bacteria and fungi. It is synthesized during fermentation process of traditional Japanese food such as miso, mirin and sake (rice wine) [Takenaka *et al*, 2000].

Properties of Glyceryl Glucoside (GG)

GG serves as osmotic balancing agents involved in the stress tolerance of living microorganisms, but also showed direct protective effects on critical macromolecules and prevented their denaturation [Hagemann & Pade, 2015]. GG exhibits better membrane protecting ability than pure polyols [Hincha & Hagemann, 2004].

GG possess excellent water solubility, high thermostability, low heat-colorability, low Maillard reactivity and high water-holding capacity [Tan *et a*l, 2016].

Topical application of glycosylglycerol was also reported to improved facial skin elasticity on human volunteers [Harada *et al*, 2010].

Formulation

Hydagen Aquaporin is compatible with all kinds of formulation, including e.g. face masks, wet wipes, foundations, BB creams, serums, ….. To easily handle the product and reduce the viscosity, it can be heated at 50°C (up to 24 hours) before the production step and at 80°C (up to 5 hours) during the formulation process.

Safety / tolerability of the product

Hydagen Aquaporin was tested to ensure its safety under the recommended conditions of use. It does not irritate the eyes or skin and no indication of skin sensitization was observed.

Description

Hydagen Aquaporin is composed by Glyceryl Glucoside (aquaporin-active moisturization booster) and Glycerin.

Composition: Glyceryl Glucoside 40-60% (and) Glycerin 40-60%

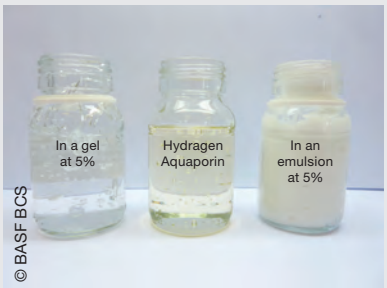
Naturalness content (according to ISO 16128): 100% from natural origin

Dose of use: 1.5-5%

China IECIC listed

Vegan Friendly **

** No involvement of animal genes or animal-derived substances; unlikely cross-contamination from animal substances; no animal testing has been carried out by or on behalf of BASF on the ingredients of the product after 11th March 2009 and/or after the extended deadline 11th March 2013 for the purposes of the Cosmetic Regulation (EU) 1223/2009 (statement available upon request).



Example: Illustration of a commercial sample of Hydagen Aquaporin, with a hydrogel and a emulsion containing 5%.

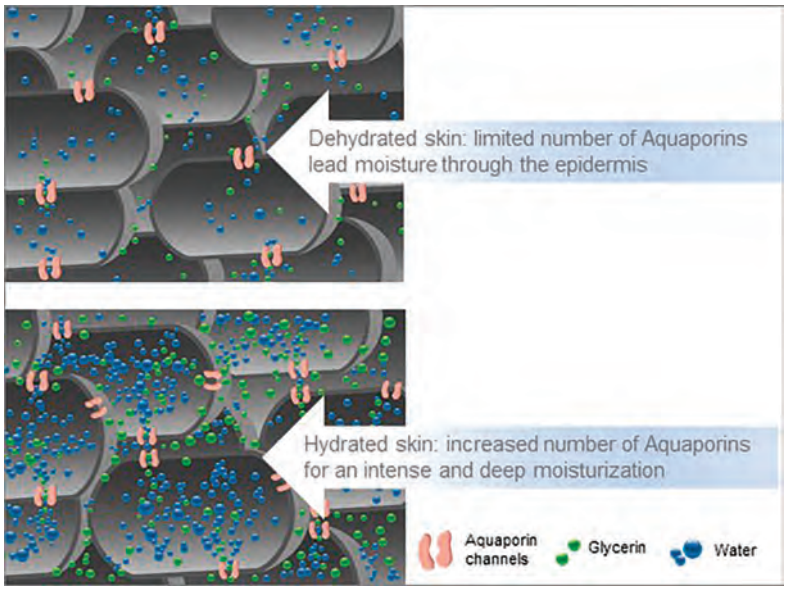
DEMONSTRATED EFFICACY

The moisturizing boosting activity of Hydagen Aquaporin was proven by *in vitro* and *in vivo* tests.

In vitro, on human epidermal keratinocytes, Hydagen Aquaporin was shown:
- to stimulate the expression of the cellular water channel protein AQP3,
- to protect AQP3 protein level in skin explants from digital blue light (additional data 2022),
- to promote the expression of involucrin protein while enhancing the cellular energy metabolism.

In vivo, Hydagen Aquaporin was shown:
- to increase AQP3 mRNA levels in the epidermis, and to improve skin barrier function [Schrader *et al*, 2012],
- to have better moisturizing effect than hyaluronic acid and glycerin after single application.
- to boost skin hydration beyond that provided by glycerin alone.

Moreover, it was demonstrated that Hydagen Aquaporin improves skin moisture and smoothness in vivo in rinse off application. Hydagen Aquaporin provides sensations of soft feel, the skin seems better protected after cleansing (additional data 2022).



EFFICACY

Upregulation of Aquaporin-3 (AQP3) protein in human keratinocytes

in vitro

OBJECTIVE

The aim of these *in vitro* assays on primary human epidermal keratinocytes was to evaluate the effect of Hydagen Aquaporin on AQP3 protein expression.

RESULTS & DISCUSSION

Human epidermal keratinocytes incubated with 1.5% Hydagen Aquaporin over a 24h period showed a significant 122% increase in AQP3 protein levels compared to the untreated control (Figure 4).

This conclusion is similar to that of Schrader and coworkers for Glyceryl Glucoside [Schrader *et al*, 2012]. *In vitro*, using immunohistochemical labelling of AQP3 protein and flow cytometry, the authors reported a 156% increase in AQP3 protein levels on normal human epidermal keratinocytes treated 24h with 3% Glyceryl Glucoside compared to untreated control.

Additionally, the authors have shown the stimulation of AQP3 mRNA expression compared to glycerin (medium of similar osmolarity) and untreated control. This upregulation of AQP3 mRNA level was also shown on a study on human subjects (cf p. 11).

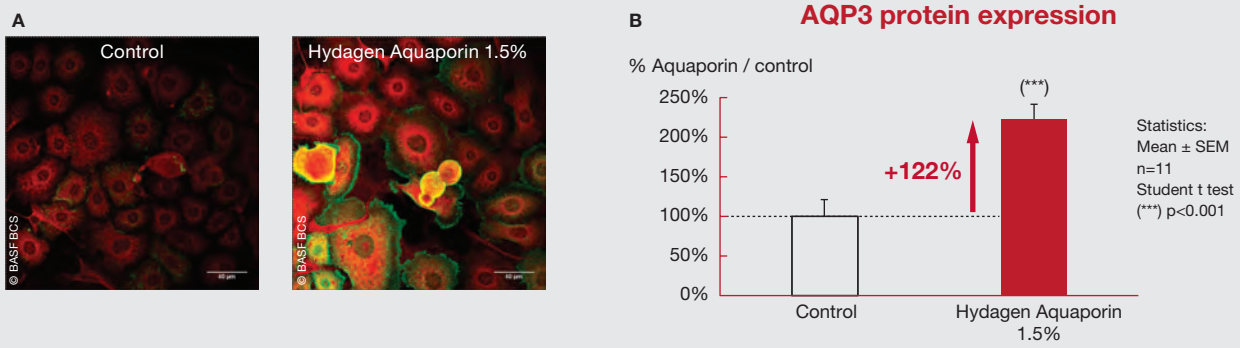


Figure 4 - AQP3 protein expression in keratinocytes after 24h of incubation with 1.5% Hydagen Aquaporin *vs* untreated control. **A** - Representative immunocytochemical pictures (AQP3 labelled in green). **B** - Quantification by image analysis.

CONCLUSION

+122% Water channel protein **Aquaporin-3**

Hydagen Aquaporin has shown *in vitro* its ability to upregulate the formation of aquaporin-3 in epidermal cells, and thus its potential to strengthen the skin's natural moisture network and to enhance its moisturization.

MATERIALS&METHODS

Cell culture and treatment

Normal human epidermal keratinocytes (NHEKs, from 30, 51 and 56 year-old women, abdomen) were seeded on collagen-coated glass slides at $3x10^5$ cells/cm² and incubated in MCDB153 (Sigma-Aldrich, France) with 2% Fetal Calf Serum (FCS), and cultured at 37°C with 5% CO_2 under 95% of relative humidity until confluence (5 days). Then the culture medium was replaced by fresh standard medium (without FCS) without (control) or with Hydagen Aquaporin at 1.5%. $CaCl_2$ 1 mM was used as positive control and validated the assays (data not shown). The absence of cytotoxicity of Hydagen Aquaporin was previously determined in an independent MTT assay (data not shown).

AQP3 immunostaining and image analysis

The culture medium was removed and the cells were quickly rinsed with PBS. After fixation with acetone for 10 minutes at -20°C and washes, the cells were incubated with the primary antibody: anti-AQP3. After several washes, the cells were incubated with the secondary antibody labelled with Alexa 488. Then, the cells were washed with PBS and counterstained by Evans blue. AQP3 fluorescence was recorded using confocal laser scanning microscope and quantified by image analysis (ratio of immunolabelled surface on total cells surface).

Statistics

The results are expressed as a percentage, as the mean ± SEM (n=11) compared to the untreated control standardized to 100%. The statistical analysis was done using Student t test according to Sigmaplot software recommendation (Systat Software Inc, USA). The threshold of significance was set to 5% ($p<0.05$).

EFFICACY

Protection of AQP3 protein level in skin explants from digital blue light (additional data 2022)

in vitro

OBJECTIVE

The aim of the study was to evaluate the protective effect of Hydagen aquaporin on in epidermis against digital blue light on AQP3 production in skin biopsies.

The blue light spectrum (400 – 500 nm) is the most energetic part of the visible light. As visible rays can penetrate deeper into the skin, accumulation of blue light exposure from the sun or from electronic devices can have deleterious impacts on the skin, particularly in the dermis and leads to premature aging and wrinkles (Nakashima *et al.* 2017). Moreover, in the skin, AQPs are known for their capacity to selectively regulate water permeability, playing a role in skin hydration. Thus, down-regulation of AQP3 may be the cause or consequence of skin dehydration through the skin glycerol content reduction and water holding capacity diminution (Hara *et al.*, 2003).

RESULTS & DISCUSSION

Results presented in figure 5 showed that Digital light reduced significantly by 78% de AQP3 protein in skin explants and that Vitamin E (positive control) was able to reverse the effect of Digital light by a protective effect of 60%. Furthermore, results showed that topical application of Hydagen Aquaporin at 1.5% protected significantly by more than 100% de AQP3 protein decreased in Digital light-stressed skin explants.

Using immunostaining we succeeded to evidence the major impact of blue light on skin AQP3 protein level.

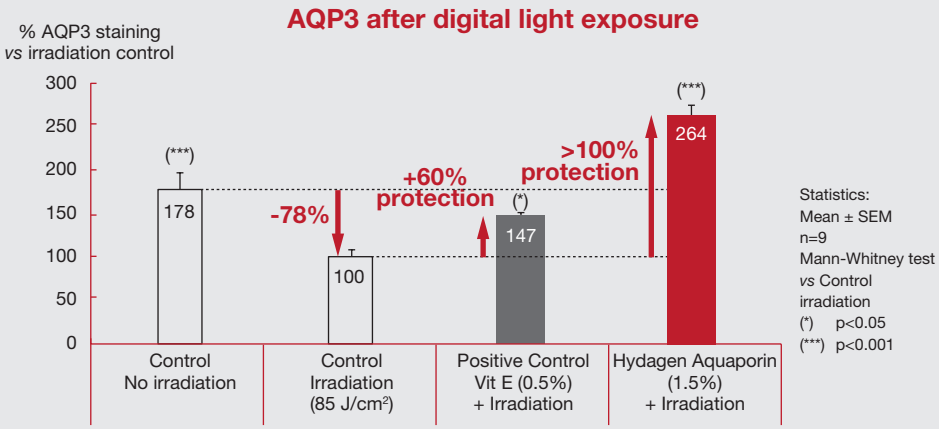


Figure 5 - AQP3 protein staining after Digital light exposure with or without Hydagen aquaporin 1.5%.

In addition, immunostaining of AQP3 protein in the epidermal layer of the skin (Figure 6A) visually demonstrated the disappearance of the pink staining with digital blue light exposure (Figure 6B). Conversely, treatment with Hydagen aquaporin 1.5% visually demonstrated the protective effect of Hydagen aquaporin of the skin against Digital blue light effect on the hydration biomarker AQP3 (Figure 6C).

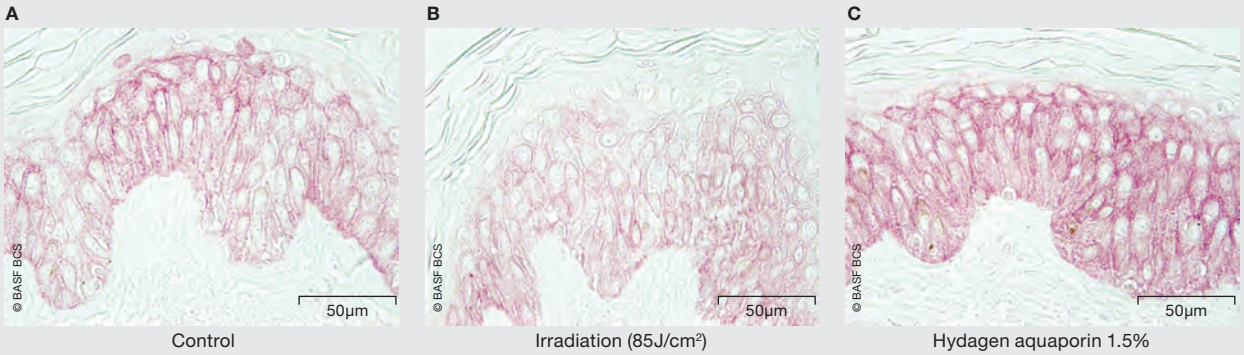


Figure 6 illustrates the visual decrease of AQP3 protein staining (pink) in Digital light-stressed skin explants **(B)** and the protective effect of Hydagen aquaporin treatment **(C)** compared to the untreated control **(A).**

CONCLUSION

Hydagen aquaporin helps to offer more than 100% protection against Digital blue light effect on the skin hydration biomarker (AQP3)

MATERIALS&METHODS

Human skin explants of an average diameter of 12 mm (±1mm) were prepared from an abdominoplasty coming of a 55-year-old caucasian woman. The explants were kept in survival in culture medium at 37°C in a humid, 5 %-CO_2 atmosphere. Hydagen Aquaporin at 1.5% or vitamin E at 0.5% were topically applied on day 0 (D0), D1 and D4 (1 hour before blue light irradiation). Then, the explants were irradiated with digital light using the Solarbox device for 4h at a dose of 85 J/cm². Then, irradiated samples and controls were cultured for 24h.

For histological analysis, samples were fixed in buffered formalin solution for 24h before dehydration and impregnation in paraffin. Then, 5-µm-thick sections were made using a Leica RM 2125 Minot-type microtome, and the sections were mounted on Superfrost histological glass slides. AQP3 immunostaining was performed on formol-fixed paraffin-embedded (FFPE) skin sections with a polyclonal anti-AQP3 antibody (Chemicon) diluted at 1:1000 in PBS-BSA 0.3%-Tween 20 0.05% and incubated for 1 hour at room temperature using a Vectastain Kit Vector amplifier system avidin/biotin, and revealed by VIP, a substrate of peroxidase giving a violet signal once oxidized (Vector laboratories). The immunostaining was assessed by microscopical observation and semi-quantified by image analysis using a Leica DMLB, Olympus BX43 or an Olympus BX63 microscope. Pictures were acquired with a numeric DP72 or DP74 Olympus camera with cellSens (Olympus) storing software.

Results and statistics

The results were normalized to the untreated control (UC) or control irradiation exposure conditions and expressed as the mean (%) ± SEM in n=9. All data has been analyzed to confirm the normality of the distribution using the Shapiro-Wilk Test.

The statistical analysis were done *vs* the irradiation control . Mann and Whitney test was used and the threshold of significance was set at 5% ($p<0.05$).

EFFICACY

Stimulation of involucrin protein expression in human keratinocytes

in vitro

OBJECTIVE

Involucrin is a protein produced by keratinocytes of early spinous layers up to granular layer of human epidermis [Eckert *et al*, 2004]. During differentiation of keratinocytes into corneocytes, involucrin is polymerized with other proteins to form the cornified envelope deposited on the inner face of cell membrane. Then transglutaminase enzymes catalyze the binding of involucrin to the γ-hydroxyceramides of lamellar lipid layers embedding the corneocytes [Proksch *et al*, 2008]. Owing to its central role in stratum corneum cohesion, involucrin represents a key element of skin barrier function.

ATP is considered to be the molecular unit of intracellular energy currency and is mainly produced by mitochondria. Hydrolysis of ATP generates energy to ensure cell metabolism and biological processes and serves as a cofactor of enzymes for signal transduction [Brand *et al*, 2013]. However, some stimulators of epidermal cell differentiation and involucrin synthesis, such as Ca^{2+} or FK866, decrease the synthesis of ATP [Tan *et al*, 2019].

The aim of this *in vitro* study on human cultured keratinocytes was to evaluate the effect of Hydagen Aquaporin on the synthesis of involucrin, without negative effect on cellular ATP level, and thus to evaluate its potential to stimulate the differentiation of human keratinocytes into corneocytes and therefore the formation of an optimal skin barrier.

RESULTS & DISCUSSION

Calcium chloride ($CaCl_2$) at 0.3 mM, used as positive control, significantly increased the synthesized involucrin by keratinocytes (+117% *vs* control, p<0.001) and decreased the level of cellular ATP (-9%, *vs* control p<0.05). These expected results validate the assay.

Hydagen Aquaporin at 1.5% significantly increased by +129% (p<0.001) the synthesis of involucrin by keratinocytes compared to the untreated control (Figure 7). Remarkably, it also showed a significant increase of cellular level of ATP (+22%, p<0.001) whereas an opposite effect is generally observed with products stimulating keratinocytes differentiation and involucrin synthesis (such as observed with the positive control $CaCl_2$ at 0.3 mM).

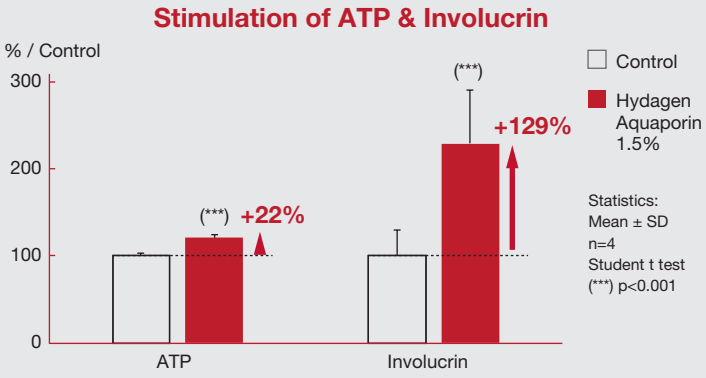


Figure 7 - ATP and involucrin cellular level in keratinocytes after 3 days of incubation with 1.5% Hydagen Aquaporin *vs* untreated control.

CONCLUSION

+22% ATP

+129% Skin barrier protein Involucrin

Hydagen Aquaporin has shown *in vitro* its ability to stimulate the synthesis of involucrin by human keratinocytes, while enhancing cellular energy metabolism, and thus its potential to stimulate their differentiation and the improvement of skin barrier function.

MATERIALS&METHODS

Cell culture and treatment

Normal human keratinocytes obtained from human skin biopsy (abdomen) from a 51-year-old woman were seeded in a defined medium (MCDB153, Sigma-Aldrich, France) with 2% Fetal Calf Serum (FCS), and cultured at 37°C with 5% CO_2 under 95% of relative humidity during 3 days. Then the culture medium was replaced by fresh standard medium (without FCS) containing 0.1 mM $CaCl_2$ without (control) or with Hydagen Aquaporin at 1.5%. $CaCl_2$ at 0.3 mM was used as positive control. The absence of cytotoxicity of Hydagen Aquaporin was previously determined in an independent MTT assay (data not shown).

Involucrin and ATP dosage

After 3 days of incubation, the cultured medium was removed, the cells were harvested and lysed with specific lysis buffer. The cellular ATP was quantified by measuring the light produced through its reaction with the Luciferase-luciferin system using a Luminometer following the instructions of manufacturer (ATP Bioluminescence Assay Kit CLS II, Roche Molecular Diagnostics, USA). The cellular involucrin was measured using an ELISA kit (BT-650, BTI Cliniscience, France) according the manufacturer instructions.

Statistics

The results are expressed as a percentage, as the mean ± SD (n=4) compared to the untreated control standardized to 100%. The statistical analysis was done using Student t test according to Sigmaplot software recommendation (Systat Software Inc, USA). The threshold of significance was set to 5% (p<0.05).

EFFICACY

Skin barrier restoration [Schrader *et al*, 2012]

in vivo

OBJECTIVE

Schrader and coworkers have evaluated whether a Glucosyl Glucoside containing formulation applied topically to volunteer could upregulate AQP3 mRNA expression and improve skin barrier function.
Two *in vivo* studies were conducted on forearms of human volunteers with a Glyceryl Glucoside containing formulation and a glycerin vehicle formulation, respectively on 19 and 23 volunteers, to determine the effects on AQP3 mRNA expression (RT-PCR) and on the barrier function (measurement of TEWL). In the first clinical study the products were applied for one week, and in the second study for 3 weeks, twice a day in both cases. An untreated area was used as control.

RESULTS & DISCUSSION

After one week of use, Glyceryl Glucoside at 5% was shown to induce a significant ($p<0.05$) increase in AQP3 mRNA expression in the epidermis, compared with the glycerin vehicle (data not shown). The authors also demonstrated a penetration of Glyceryl Glucoside in the epidermis.

Compared with the untreated control area, Glyceryl Glucoside at 5% induced a significant ($p<0.05$) decrease in TEWL by 10% after the 3 weeks of treatment. This decrease of water loss is 2.2 times better ($p<0.05$) than the one observed with the glycerin vehicle alone (Figure 8), demonstrating an improved skin barrier function.

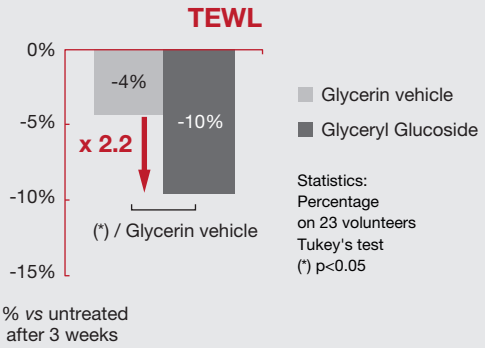


Figure 8 - Effect on skin barrier function. Difference of percentage change in TEWL from baseline between the tested product and the untreated area. Glycerin Glucoside at 5% *vs* glycerin vehicle.

CONCLUSION

x2.2 Reduction of TEWL *vs* glycerin vehicle — After 3 weeks

Through *in vivo* studies, Schrader and coworkers have shown that Glyceryl Glucoside at 5% increased AQP3 mRNA levels in the skin and reduced the transepidermal water loss compared with glycerin vehicle.

MATERIALS&METHODS

AQP3 mRNA levels in response to Glyceryl Glucoside treatment

Study design

The clinical study was carried-out double-blind, with application by the volunteers of the products on defined areas on the inner forearms for 7 days, and a untreated area. Two products were evaluated, a glycerin vehicle formulation (based on PEG-40 stearate and 6.5% glycerin) containing Glyceryl Glucoside at 5%, and the same glycerin vehicle formulation without Glyceryl Glucoside. The AQP3 mRNA expression was measured after the 7 days of the study.

Inclusion criteria

The study was done on 19 healthy male and female volunteers aged from 22 to 53 years old.

Application modality

Volunteers applied realistic amounts of the products twice daily to the defined areas of the inner arms over the 7 days period. No product was applied in the morning prior AQP3 mRNA expression analysis, ensuring that at least 15h had elapsed since the last application of test formulations.

Evaluation method

Total RNAs were isolated from skin suction blister on treated areas for RT-PCR analysis. The levels of AQP3 mRNA expression were normalized to the respective levels of the housekeeping gene 18S rRNA and the results expressed as change in AQP3 mRNA expression relative to the untreated (control) area.

Skin barrier function in response to Glyceryl Glucoside treatment

Study design

The clinical study was carried-out double-blind, with application by the volunteers of the products on defined areas on the inner forearms for 3 weeks, and a untreated area. Two products were evaluated, a glycerin vehicle formulation (based on PEG-40 stearate and 6.5% glycerin) containing Glyceryl Glucoside at 5%, and the same glycerin vehicle formulation without Glyceryl Glucoside. The skin barrier function (Trans Epidermal Water Loss, TEWL) was evaluated at baseline and after the 3 weeks of the study.

Inclusion criteria

The study was done on 23 healthy female volunteers aged from 50 to 70 years old, with dry skin (TEWL values between 5 and 9 g/m²/h).

Application modality

Volunteers applied realistic amounts of the products twice daily to the defined areas of the inner arms over the 3 weeks period. No product was applied in the morning prior to measurement.

Evaluation method

Skin barrier function measurements were done with a Tewameter TM300 (Courage + Khazaka, Cologne, Germany) which determines the TEWL (g/m²/h). Five measurements were performed on each test area (treated and untreated) and the mean was used to define the TEWL. The decrease of the TEWL shows less water loss. The results are expressed as the difference of percentage change in TEWL from baseline between the tested product and the untreated area: $[(Tn\text{-}T0)/T0]_{Tested\ product}$ - $[(Tn\text{-}T0)/T0]_{Untreated}$.

Statistics

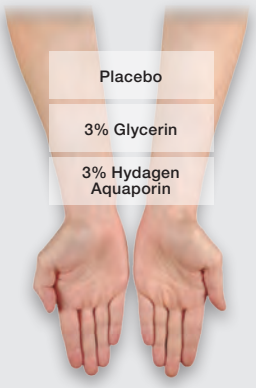
One-way analysis of variance with post-hoc comparisons using Tukey's test was used to compare data among groups. The threshold of significance was set to 5% ($p<0.05$).

EFFICACY
24h moisturizing effect

in vivo

OBJECTIVE

In a double-blind placebo-controlled clinical study on human volunteers, we evaluated the 24h moisturizing effect of Hydagen Aquaporin *vs* glycerin (Figure 9).



- 40 volunteers, over 18 years old, with dry skin on the inner side of the forearm.
- Single application on the forearms of 3% Hydagen Aquaporin in O/W emulsion *vs* Glycerin and placebo.
- Corneometer measurements (skin capacitance) before and up to 24 h after application.

Figure 9 - Clinical study on 24h moisturizing effect of Hydagen Aquaporin *vs* Glycerin and *vs* Placebo.

RESULTS & DISCUSSION

Hydagen Aquaporin at 3% and glycerin at 3% have both demonstrated a similar moisturization effect up to 8h after a single application (similar significant increase of skin capacitance versus the placebo control, data not shown).

But 24 hours after a single application, only Hydagen Aquaporin at 3% demonstrated a moisturizing effect whereas glycerin at 3% and placebo control have no more any effect (Figure 10). This increase of skin capacitance was significantly better than glycerin at 3% and placebo control.

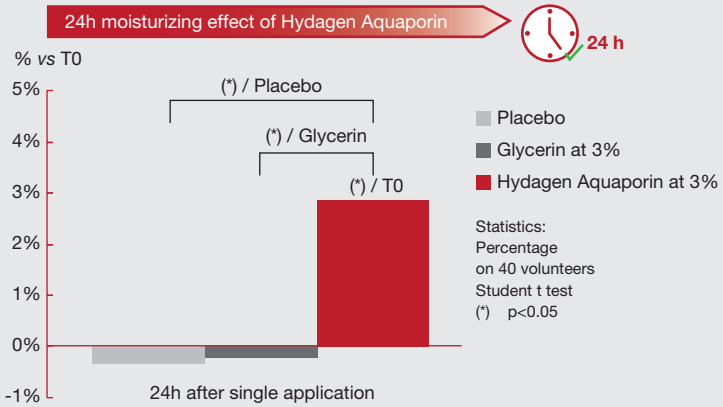


Figure 10 - 24h skin moisturizing effect of Hydagen Aquaporin after a single application. Corneometer values change *vs* baseline (T0) for Hydagen Aquaporin 3%, Glycerin 3% and Placebo O/W emulsion.

CONCLUSION

Better than glycerin After 24 hrs

In vivo, Hydagen Aquaporin at 3% significantly improved the skin moisturization after a single application. Comparatively to glycerin at the same dose, only Hydagen Aquaporin at 3% had a moisturizing effect lasting 24h after a single application.

This shows for Hydagen Aquaporin a better 24h moisturizing effect than glycerin.

MATERIALS&METHODS

Study design

The clinical study was carried-out double-blind versus benchmark, glycerin, and versus placebo, with standardized application of the products on defined areas on the forearms. The efficacy of the O/W emulsion containing Hydagen Aquaporin at 3%, glycerin at 3% or the placebo were compared at 2, 8 and 24h. The O/W emulsion formulation is detailed in Annex 2.

Inclusion criteria

The study was done on 40 healthy female volunteers aged from 18 to 65 years old, with dry skin on the inner side of the forearms (corneometer value ≤ 40 a.u.).

Application modality

A single standardized application (2 mg/cm²) of the products was done by a technical expert.

Evaluation method

Skin hydration measurements were done with a Corneometer MPA 5 CPU (Courage + Khazaka, Cologne, Germany) which registers the electrical capacitance of the skin surface. The capacitance is expressed digitally in arbitrary units (a.u.). Five measurements were performed on each test area and the mean was used to define the hydration state of the skin. The increase in the parameter shows a moisturizing effect of the product. The results are expressed as the percentage of evolution *vs* baseline, (Tn-T0)/T0, for each product.

Statistics

The statistical analysis of the evolution of the parameters as a function of time has been done after the verification of the normality of distribution using Shapiro-Wilk test. The statistical analysis of the change in the studied parameters for each product over time as well as the differences in the studied parameters between the treatment groups were performed as follows:

- validation of the normality of the studied parameters: paired Student t test,
- invalidation of the normality of the studied parameters: Wilcoxon test.

The threshold of significance was set to 5% ($p<0.05$).

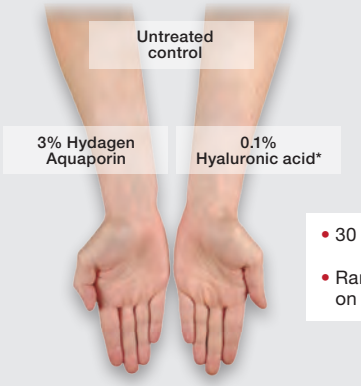
EFFICACY

Skin hydration versus hyaluronic acid (additional data 2022)

in vivo

OBJECTIVE

The hydration effect of Hydagen Aquaporin at 3% was tested in a clinical study on human volunteers versus hyaluronic acid at 0.1%, Glycerin at 3%, and the control (area without treatment).
The study was done on 30 Asian female volunteers presenting a dry skin on the forearm. The treatment was applied once at D0 and the hydration level was evaluated by Corneometer before, 2, 4, 8 and 24 hours after one application (Figure 11).



- 30 Chinese volunteers 20-45 years old.
- Randomized, double-blind single application, on the inner side of the forearm.

** High molecular weight (1300 kDa)*

Figure 11 - Clinical study on skin hydration versus hyaluronic acid

RESULTS & DISCUSSION

2, 4 and 8 hours after a single application of Hydagen Auqaporin at 3%, the moisturizing effect is significantly better than Placebo, glycerin at 3% and hyaluronic acid at 0.1%.

24 hours after this application, the effect is significantly better than hyaluronic acid at 0.1% ($p<0.01$) and close to the significance for glycerin at 3% ($p<0.1$) (Figure 12).

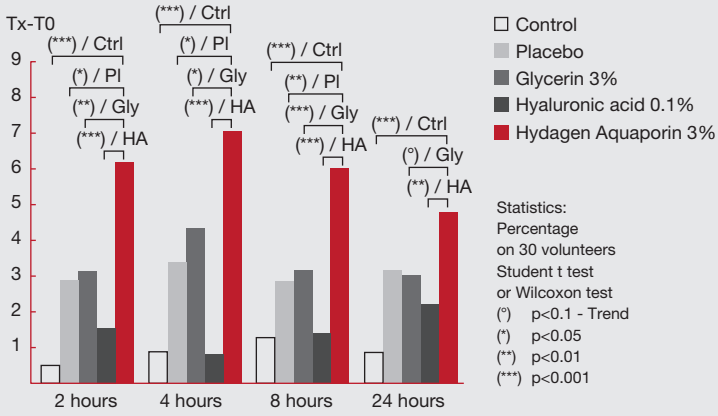


Figure 12 - Evaluation of skin hydration measured with Corneometer

CONCLUSION

Skin moisturizing effect of Hydagen Aquaporin at 3% lasted up to 24h after a single application, better than placebo, glycerin at 3% and hyaluronic acid at 0.1% at 2, 4 and 8 hours and better than hyaluronic acid at 24hours ($p<0.05$) and glycerin ($p<0.1$).

MATERIALS&METHODS

Study design
The clinical study was carried out in double-blind and randomized in China (Shanghai), after only one product application on defined areas on the forearms, hydration was measured with Cutometer at baseline and after 2, 4, 8 and 24 hours.

Inclusion criteria
The study was done on 30 healthy Chinese women, age between 18 and 45 years old presenting a dry skin on the forearm.

Application modality
Hydagen Aquaporin at 3% and Hyaluronic Acid at 0.1% and glycerin at 3%, were applied once at D0 on volar forearm at laboratory. The formulas were detailed in Annex 2.

Evaluation method
Corneometry is a technique used to determine the level of moisture in the outer layers of the stratum corneum. This method is based on the relationship between the electrical properties of skin tissues and their moisture content. The principle of corneometry consists in passing a high frequency electric current through the skin between two electrodes. The electric field produced in the epidermis is function to the geometry and the dielectric constant of the electrodes and of the capacitance of the skin in contact with the probe. A variation in the moisturization of the skin is traduced by a modification of the total capacitance of the system. The device used is a CM 825 (Courage and Khazaka, Germany).
Values are expressed in arbitrary units as the difference Tn-T0 for each product.
The increase in the parameter shows a moisturizing effect of the product.

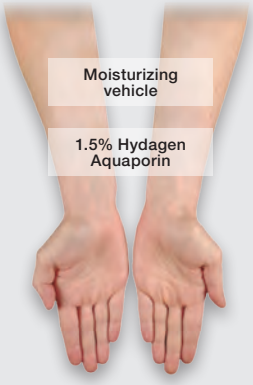
Statistics
The statistical analysis of the parameters has been done after the verification of the normality of distribution using Shapiro-Wilk test
Afterwards, the statistical analysis was performed with the Student t-test in case of the normality of distribution had been confirmed. In case of the distribution had not followed the normal law, a non-parametric test (Wilcoxon rank test) was used.

EFFICACY
Hydration booster

in vivo

OBJECTIVE

In a double-blind clinical study on human volunteers, the hydration boosting effect of Hydagen Aquaporin at 1.5% formulated in a 4.5% glycerin moisturizing emulsion was evaluated *vs* the moisturizing emulsion vehicle containing the same amount of glycerin (Figure 13).



- 47 volunteers, over 18 years old, with dry skin on the inner side of the forearm.
- Single application on the forearms of 1.5% Hydagen Aquaporin in a 4.5% glycerin moisturizing vehicle *vs* the glycerin moisturizing vehicle.
- Both O/W emulsion formulations contain the same amount of Glycerin (5.3%), and differ only by the active Glyceryl Glucoside.
- Corneometer measurements (skin capacitance) before, 20 min, 6 and 24 h after application.

Figure 13 - Clinical study on moisturizing boosting effect of Hydagen Aquaporin.

RESULTS & DISCUSSION

At each time points (20 min, 6 h and 24 h after a single application) the Hydagen Aquaporin cream significantly improved the skin hydration level *vs* baseline and the glycerin moisturizing vehicle (Figure 14): 20 min, 6 h and 24 h after a single application, the Hydagen Aquaporin cream increased the skin capacitance respectively by 81%, 34% and 20% as compared to baseline.

The moisturizing boosting effect of Hydagen Aquaporin increased significantly with time: from 1.18 times more effective than the glycerin moisturizing vehicle 20 minutes after application, to 1.25 times after 6h and 1.5 times (+50%) after 24 hours (Figure 15). 24h after application, Hydagen Aquaporin increased increases 1.5x the hydration power of the glycerin moisturizing O/W emulsion. As the formulations in both cases contained the same amount of glycerin, these results prove the potency of Glyceryl Glucoside in Hydagen Aquaporin to boost skin hydration beyond that provided by glycerin, which is a widely accepted hydrating agent that is included in most moisturizing product formulations. Such an effect is similar to the findings reported by Schrader and coworkers on the properties of Glyceryl Glucoside for barrier restoration and prevention of water loss for the skin [Schrader *et al*, 2012; see p. 12].

The use of formulations containing Hydagen Aquaporin could therefore be contemplated as an additional step to have a well moisturized skin.

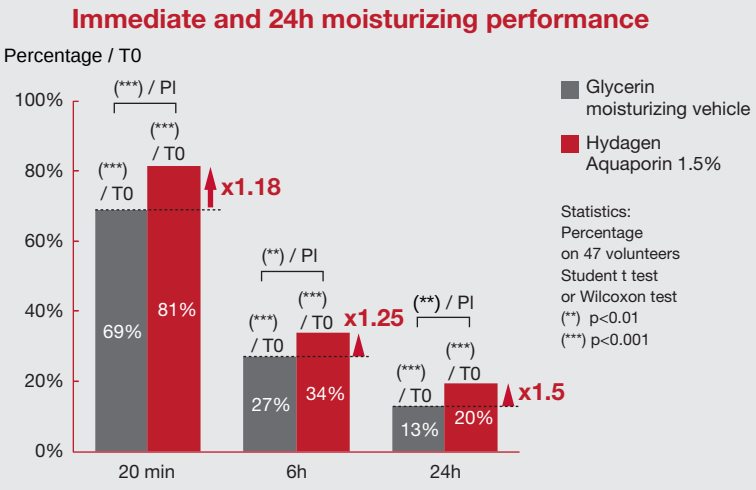


Figure 14 - Skin moisturizing effect of Hydagen Aquaporin after a single application. Corneometer values change *vs* baseline (T0) for Hydagen Aquaporin 1.5% *vs* glycerin moisturizing vehicle.

Hydration boosting effect

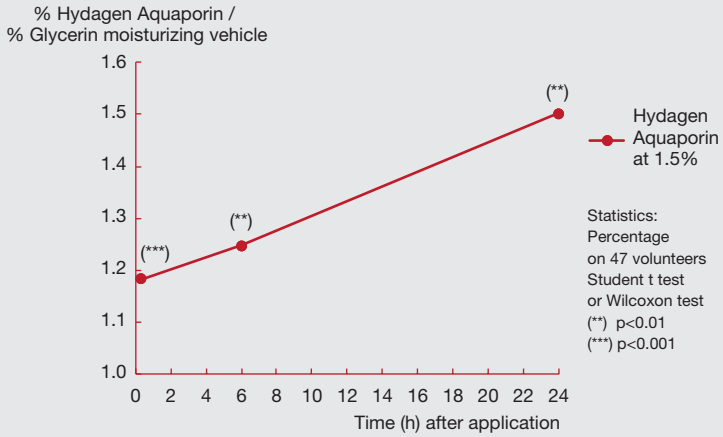
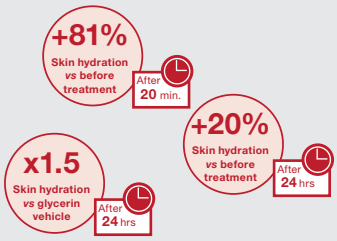


Figure 15 - Hydration boosting effect of Hydagen Aquaporin after a single application. Ratio of corneometer values change *vs* baseline (T0) for Hydagen Aquaporin 1.5% / glycerin moisturizing vehicle.

CONCLUSION



In vivo, Hydagen Aquaporin at 1.5% formulated in a 4.5% glycerin moisturizing emulsion significantly improved the immediate and 24 hours skin moisturization after a single application, with a significantly better effect than the moisturizing emulsion containing the same amount of glycerin.

Hydagen aquaporin at 1.5% had a moisturizing boosting effect increasing with time, up to 1.5 time more effective than the moisturizing emulsion 24h after the application.

This shows the capability of Hydagen Aquaporin to boost skin hydration beyond that provided by glycerin alone.

MATERIALS&METHODS

Study design

The clinical study was carried-out double-blind, with standardized application of the products on defined areas on the forearms. Two products were evaluated, an O/W emulsion containing Hydagen Aquaporin at 1.5% and glycerin at 4.5%, and the same emulsion (glycerin moisturizing vehicle) containing the same amount of glycerin (considering the amount of glycerin from Hydagen Aquaporin), the products differing only by the active Glyceryl Glucoside. The O/W emulsion formulations are detailed in Annex 2. The skin moisturization was evaluated 20 min, 6 and 24h after product application.

Inclusion criteria

The study was done on 47 healthy female volunteers aged from 18 to 65 years old, with dry skin on the inner side of the forearms (corneometer value ≤ 45 a.u.).

Application modality

A single standardized application (2mg/cm²) of the products was done by a technical expert.

Evaluation method

Skin hydration measurements were done with a Corneometer MPA 5 CPU (Courage + Khazaka, Cologne, Germany) which registers the electrical capacitance of the skin surface. The capacitance is expressed digitally in arbitrary units (a.u.). Five measurements were performed on each test area and the mean was used to define the hydration state of the skin. The increase in the parameter shows a moisturizing effect of the product. The results are expressed as the percentage of evolution *vs* baseline, (Tn-T0)/T0, for each product, and as the ratio $[(Tn-T0)/T0]_{Hydagen\ Aquaporin}$/ $[(Tn-T0)/T0]_{Glycerin\ vehicle}$.

Statistics

The statistical analysis of the evolution of the parameters as a function of time has been done after the verification of the normality of distribution using Shapiro-Wilk test. The statistical analysis of the change in the studied parameters for each product over time as well as the differences in the studied parameters between the treatment groups were performed as follows:

- validation of the normality of the studied parameters: paired Student t test,
- invalidation of the normality of the studied parameters: Wilcoxon test.

The threshold of significance was set to 5% ($p<0.05$).

EFFICACY

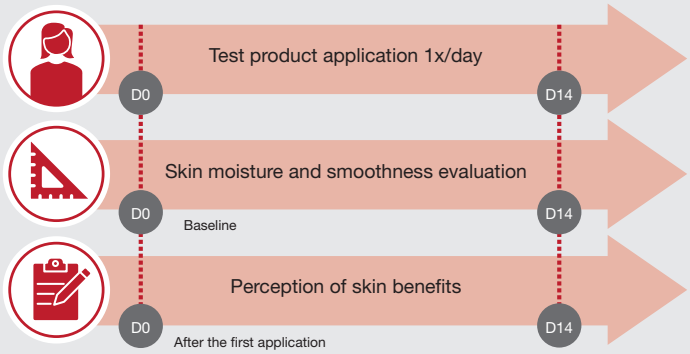
Skin hydration and smoothness in rinse off formula (additional data 2022)

in vivo

OBJECTIVE

The moisturization and smoothing efficacy of Hydagen Aquaporin at 3% was evaluated in a rinse-off formulation in a double-blind, placebo-controlled and split-body study (Figure 16).
A consumer self-perception questionnaire was administered to subjects immediately following the first usage and after 14 days of daily use.

- 27 female volunteers over 18 years old, having self-perceived dry/itchy/uncomfortable skin and having dry skin.
- Self-application by volunteers once daily of 3% Hydagen Aquaporin or placebo in a cleanser for 14 days. The product was left on their skin for 60s before rinsing the product off.



Epsilon measurements (skin capacitance and roughness) at D0 and D14

Figure 16 - Clinical study on moisturizing and smoothing effect of Hydagen Aquaporin in a cleanser (rinse-off formulation)

RESULTS & DISCUSSION

Skin moisture

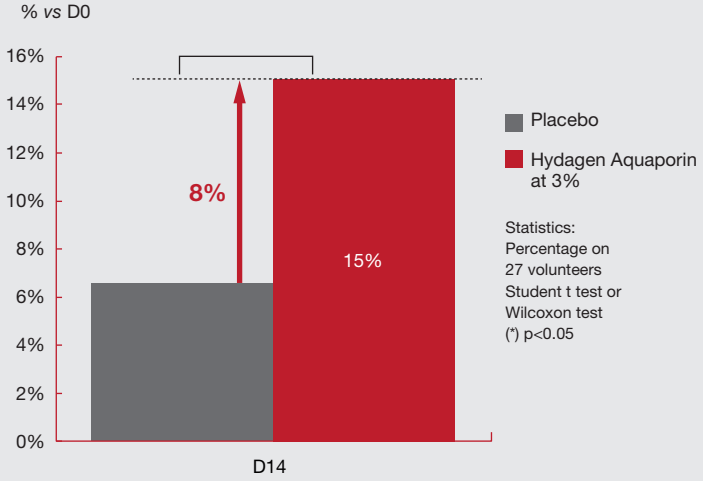
After 14 days of application, Hydagen Aquaporin showed a significant ($p<0.05$) increase of 15% in Epsilon value (average dielectric constant) as compared to baseline, as well as a significant ($p<0.05$) increase of 8% in average dielectric constant as compared to the placebo (Figure 17).

An increase in the skin's dielectric constant is caused by an increase in the skin's moisture level.

Therefore, these results demonstrate the ability of Hydagen Aquaporin used at 3% in a rinse off formula to increase the skin's moisture levels after 14 days of use.

Figure 17 – Skin hydration effect of Hydagen Aquaporin after 14 days of daily application in a cleanser. Epsilon value (average dielectric constant) changes at D14 *vs* D0 for Hydagen Aquaporin 3% *vs* Placebo.

Epsilon: Dielectric Constant - Skin Hydration



Skin roughness

These results show that Hydagen Aquaporin at 3% in a rinse-off formula leads to a decrease in skin roughness of 15% compared to the Placebo ($p<0.01$), after 14 days of use (Figure 18).

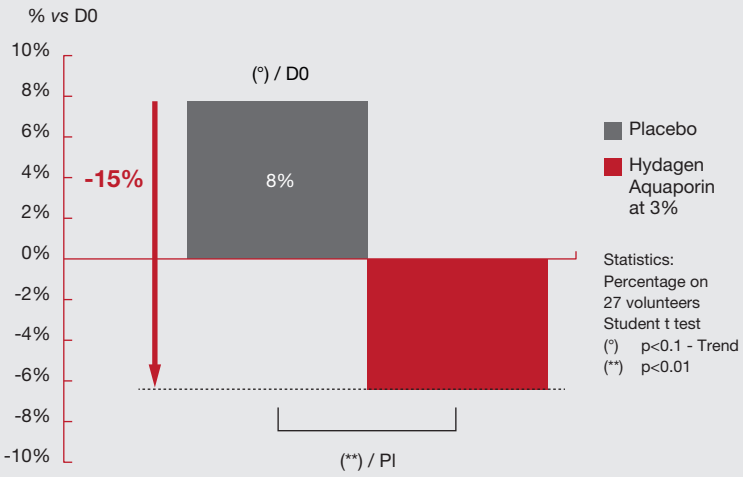


Figure 18 – Effect of Hydagen Aquaporin on skin roughness after 14 days of daily application. Epsilon value (percent pixels in non-contact range) changes at D14 *vs* D0 for Hydagen Aquaporin 3% *vs* Placebo.

Illustrative Epsilon's images

Representative Images in Figure 19 illustrate the moisturizing effect of Hydagen Aquaporin at 3%. Brighter pixels represent a higher dielectric constant and therefore a higher moisture level. The images also illustrate the smoothing effect of Hydagen Aquaporin, with a reduction of darker pixels representing skin in microrelief.

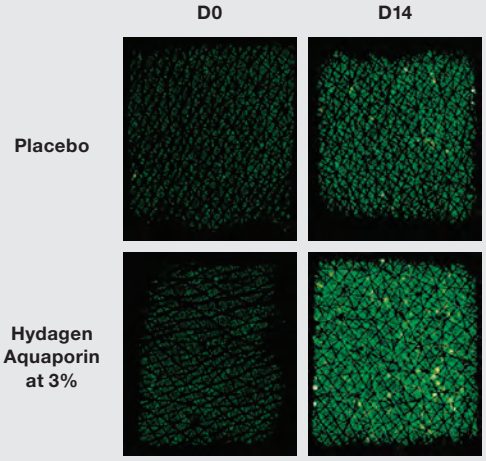


Figure 19 – Illustrative Images for Skin Moisturization and Smoothness. In Green: Color and brightness of the pixel is related to the level of skin hydration, with brighter pixels representing greater skin moisture. In Black: dark pixels represent areas of the skin out of contact with the probe caused by skin microrelief.

Consumer perception

Hydagen Aquaporin was perceived by a significant majority ($p<0.05$) of volunteers as leaving skin feeling softer immediately after one use (Figure 20). After 14 days of daily use, Hydagen Aquaporin was rated significantly higher than the placebo for leaving skin better protected" (Figure 21).

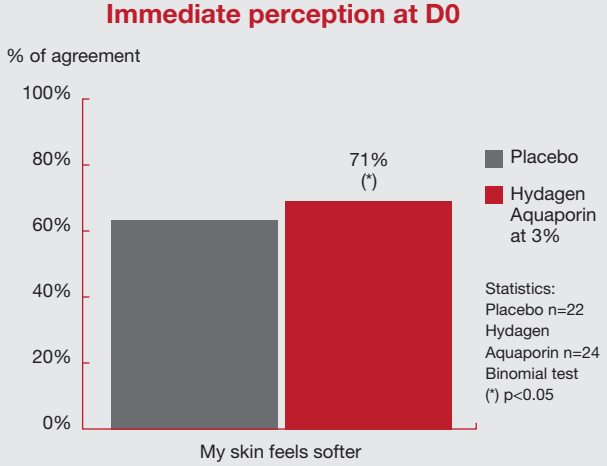


Figure 20 – Immediate consumer's self-perception assessment on skin benefits after the first application of the rinse-off formula containing or not (placebo) Hydagen Aquaporin at 3%.

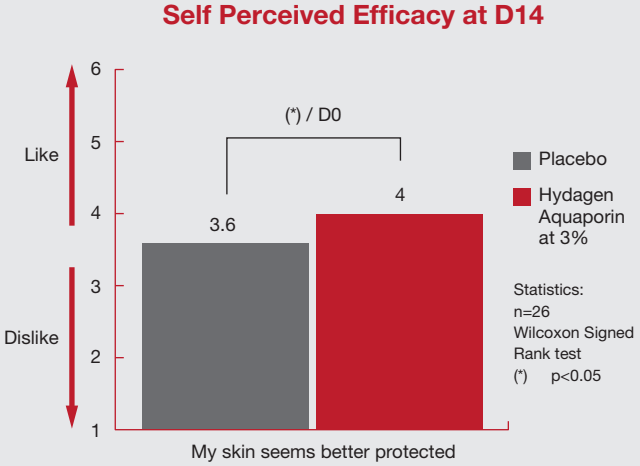


Figure 21 – Consumer's self-perception assessment on skin benefits after 14 days of daily use of the rinse-off formula containing or not (placebo) Hydagen Aquaporin at 3%. Evaluation scale: Disliked: (1) Disagree Strongly, (2) Disagree Somewhat, (3) Disagree Slightly; Liked: (4) Agree Slightly, (5) Agree Somewhat, (6) Agree Strongly.

These results demonstrate that Hydagen Aquaporin is able to produce perceptible skin benefits for the consumer on skin softening and protection when used at 3% a rinse-off formulation.

CONCLUSION

Hydagen Aquaporin at 3% shows both skin moisturization and skin smoothing efficacy after 14 days of use in a rinse-off product and provides perceivable benefits immediately and over time.

MATERIALS&METHODS

Study design

A double-blind, placebo controlled, split-body and randomized study was conducted to evaluate the effect of Hydagen Aquaporin on skin moisturization and smoothness in rinse-off cleansing formula. The application of the cleansers on defined areas on the left and right forearms was standardized. Evolution of the parameters was compared to prior to treatment (D0), and versus the placebo formulation.The rinse-off formulation is detailed in Annex 2.

Inclusion criteria

The study was done on 27 female volunteers aged 18-65 years considering themselves to have dry/itchy/uncomfortable skin and having dry skin (Corneometer Value under 50, that corresponds to an Epsilon Average Dielectric Constant of ~17, Pan *et al.* 2014).

Application modality

Volunteers self-applied the cleanser containing Hydagen Aquaporin or the Placebo once daily for 14 days. Volunteers were instructed to wet their arms, apply two pumps of the product to their forearms, and lather the product evenly over their entire volar forearms. They were then instructed to leave the product on their skin for 60s before rinsing the product off. The last application was the day prior to Day 14.

Evaluation method

Skin hydration and texture measurements

Biox Epsilon instrument was used to provides images that can be analyzed to measure skin hydration and texture.

Epsilon can measure skin hydration with greater accuracy and flexibility by using the capacitance method. It uses a sensor array of micro-capacitors to calculate, in a spatial representation of the sample, the dielectric permittivity of the soft materials. Each pixel intensity of the image from 0-255 (0=black, 255=white) correlates linearly to dielectric permittivity. The average dielectric constant of the pixels falling in the skin moisture range (3-80) and positively correlates to the skin moisture (water content). The higher the Epsilon value, the higher the skin moisture.

The second parameter used is for skin roughness. The method was to analyze the percentage of pixels that were accounted for by pixels in the non-contact range (dielectric constant 0-3), out of the total number of pixels. The topography of the skin (furrows, ridges, and microlines) create non-contact areas, as the skin dips down away from the probe. When there are more furrows, dips or ridges in the skin, non-contact pixels will account for a greater share of the image, and the non-contact pixel percentage will increase. A decrease in the non-contact pixel percentage is associated with a reduction in roughness and a skin smoothening effect.

Consumer Self-Perception Questionnaire

The volunteers completed self-administered questionnaires to evaluate their perception of the test product's performance and the resulting skin benefits. Questionnaires were completed through Internet-based software at the test center at D0 and D14. The volunteers's responses were organized and subjected to analysis to determine statistical significance. The number of volunteers who completed the questionnaires were 22 and 24 for the placebo and the Hydagen Aquaporin cleansers immediately after the first use, and 26 for both products at the end of the study. Other data are missing.

Statistics

Instrumental Values

Results from instrumental measurements are expressed as mean percentage of evolution versus baseline (%/D0) +/- the standard deviation of the sample (SD) or the standard error of the sample (SEM). All data has been analyzed to confirm the normality of the distribution using the Shapiro-Wilk Test. Afterwards, the statistical analysis of the change of the studied parameters for each product was performed with the Student's t-test in case of confirmed normality. In case of non-normal distribution, a non-parametric test (Wilcoxon Signed Rank Test) was used.

Consumer Self-Perception Questionnaire

The volunteers self-evaluated the sensorial benefits on dry skin after each treatment. They used a 6-points scale. Possible answers were (1) Disagree Strongly, (2) Disagree Somewhat, (3) Disagree Slightly, (4) Agree Slightly, (5) Agree Somewhat, (6) Agree Strongly.

The analysis involves establishing frequency tables that take into account the number of responses and calculating the frequency of the different possible answers (given as percentage) to each qualitative question.

Two methods were used for data analysis.

For the immediate consumer's self-perception assessment on skin benefits, the efficacy and the appreciation of the products for each item were grouped as follow: two percentages Z1 and Z2 are calculated; Z1 = favorable opinion (agree) and Z2 = unfavorable opinion (disagree) (Chambers and Wolf, 1996). The statistical difference in frequencies of responses (%) between favorable (agreement) and unfavorable (disagreement) opinions was evaluated using Two-tailed binomial test.

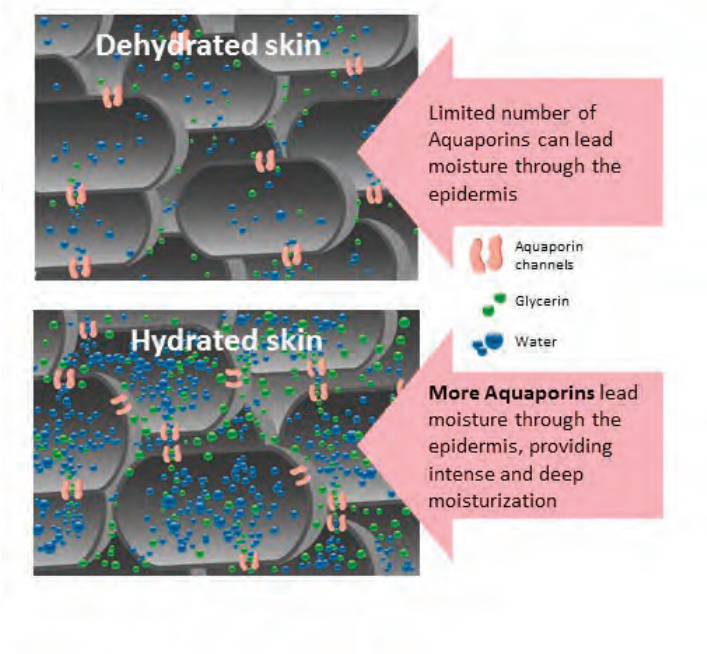
For the consumer self-perception assessment on skin benefits after 14 days of daily use, results were expressed as the mean values of the volunteers'ratings (6-points scale). The statistical differences between Hydagen Aquaporin and the Placebo were evaluated using the Wilcoxon Signed Rank Test.

The threshold for significance was set up to 5% ($p<0.05$).

GENERAL CONCLUSION

Hydagen Aquaporin, the aquaporin-active moisturization booster

- Technology inspired by a Nobel Prize-awarded discovery on cellular water channels, the aquaporins.
- The active ingredient, Glyceryl Glucoside, is a nature-identical molecule, and 100% from natural origin.
- Hydagen Aquaporin stimulates the formation and protects aquaporins in the epidermal cells.
- In clinical studies, Hydagen Aquaporin has proven to have powerful moisturization boosting effect, for hydrating the skin beyond glycerin and Hyaluronic acid.



The moisturizing active ingredient Hydagen Aquaporin is based on scientific findings from the 1990s. Back then, researchers – later awarded the 2003 Nobel Prize in Chemistry – clarified how water molecules are transported across skin cells via specialized channels. This fundamental process of life relies on integral membrane proteins called aquaporins. BASF Care Creations has now brought the aquaporin-stimulating substance glyceryl glucoside in a highly concentrated form to the broad cosmetic market as the active matter of Hydagen Aquaporin. Both *in vitro* and *in vivo* studies have proven its hydration-boosting effect.

In *in vitro* studies, Hydagen Aquaporin has shown its ability to upregulate the formation of aquaporin-3 and involucrin in epidermal keratinocytes, and to protect AQP3 from digital light effects. All these results demonstrate its potential to improve the transport of moisture in the skin as well as the skin's barrier function.

In clinical studies, Hydagen Aquaporin has proven a potent immediate moisturizing effect, better than glycerin and Hyaluronic acid at 24h, and its ability to boost skin hydration beyond that provided by glycerin alone. Used in rinse-off, it also showed benefits on skin moisturization and roughness.

Hydagen Aquaporin promotes aquaporin-3 upregulation in the epidermis, improves and boosts skin moisturization, and thus offers an effective solution for dry and damaged skin and scalp.

Hydagen Aquaporin is suitable for use in rinse off application. It improves skin moisture, smoothness and provides sensations of soft feel and skin protection after cleansing.

ANNEXES

Annex 1 - Technical data - Available upon request

- Quality and Regulatory Product Information
- Information on toxicological data
- Composition sheet
- Specifications
- Formulation Data Sheet
- Halal statement
- Natural content (according ISO 16128)
- Vegan statement

Annex 2 - Clinical test formula

24h moisturizing clinical test

Trade name	INCI name	Placebo formulation %	Glycerin formulation %	Hydagen Aquaporin fomulation %
Eumulgin B 2	Ceteareth-20	3.00	3.00	3.00
Lanette 16	Cetyl Alcohol	0.90	0.90	0.90
Lanette 18	Stearyl Alcohol	2.10	2.10	2.10
Cetiol LC	Coco Caprylate / Caprate	15.00	15.00	15.00
Rheocare C Plus	Carbomer	0.20	0.20	0.20
Water	Water	qsf. 100	qsf. 100	qsf. 100
Preservative	-	qs	qs	qs
Glycerin	Glycerin	-	3.00	-
Hydagen Aquaporin	Glyceryl Glucoside (and) Glycerin	-	-	3.00

Skin Hydration versus Hyaluronic Acid

Trade name	INCI name	Placebo formulation %	Glycerin formulation %	Hyaluronic acid %	Hydagen Aquaporin fomulation %
Chlorphenesin	Chlorphenesin	0.25	0.25	0.25	0.25
Cabopol EDT 2020	Acrylates/C10-30 alkyl acrylate crosspolymer	0.30	0.30	0.30	0.30
Phenoxyethanol	Phenoxyethanol	0.50	0.50	0.50	0.50
Butylene Glycol	Butylene Glycol	3.00	3.00	3.00	3.00
Glycerin	Glycerin	-	3.00	-	-
Hyaluronic acid	Hyaluronic acid	-	-	0.10	-
Hydagen Aquaporin	-	-	-	-	3.00
Sodium Hydroxide	Sodium Hydroxide	q.s.	q.s.	q.s.	q.s.
Aqua	Aqua	qsf 100.00			

Hydration booster clinical test

Trade name	INCI name	Placebo formulation %	Hydagen Aquaporin fomulation %
Eumulgin B 2	Ceteareth-20	3.00	3.00
Lanette 16	Cetyl Alcohol	0.90	0.90
Lanette 18	Stearyl Alcohol	2.10	2.10
Cetiol LC	Coco Caprylate / Caprate	15.00	15.00
Rheocare C Plus	Carbomer	0.20	0.20
Water	Water	qsf. 100	qsf. 100
Preservative	-	qs	qs
Glycerin	Glycerin	5.30*	4.50*
Hydagen Aquaporin	Glyceryl Glucoside (and) Glycerin	-	1.50

** Both O/W emulsion formulations contains the same amount of glycerin (5.3%), and differs only by the active Glyceryl Glucoside.*

Cleansing clinical test (rinse off application)

Trade name	INCI name	Placebo formulation %	Hydagen Aquaporin fomulation %
Deionized Water	Water	71.20	68.20
Sodium Benzoate	Sodium Benzoate	0.50	0.50
Rheocare® XGN	Xantham Gum	0.80	0.80
Plantapon® ACG50	Sodium Cocoyl Glutamate	10.00	10.00
Plantacare® 2000 UP	Decyl Glucoside	13.00	13.00
Lamesoft® PO 65	Coco-Glucoside, Glyceryl Oleate	1.00	1.00
Sol. Citric Acid 50%	Citric Acid	3.50	3.50
Hydagen® Aquaporin	-	-	3.00

Annex 3 - Formulation examples

Facial Cleansing Mousse HB-CN-20-CL061201

Phase	Ingredients	INCI	% by weight	Function
A	Plantacare® 2000 UP Lamesoft® PO 65 Water, demin.	Decyl Glucoside Coco-Glucoside, Glyceryl Oleate Aqua	13.00 2.00 68.20	Surfactant Conditioning agent
B	Plantapon® ACG 50 Hydagen® Aquaporin Glycerin	Sodium Cocoyl Glutamate Glyceryl Glucoside, Glycerin Glycerin	10.00 1.00 4.00	Surfactant Active ingredient Humectant
C	Citric Acid Sodium Benzoate	Citric Acid Sodium Benzoate	1.30 0.50	pH Adjustment Preservative

Specifications	
pH value (23°C)	about 5.15
Appearance	Transparent liquid

Jammin' Jelly Body Wash HB-US-21-36149-30

Phase	Ingredients	INCI	% by weight	Function
A	Water, demin. Cosmedia® Ultragel 300	Aqua Polyquaternium-37	70.00 4.50	 Rheology modifier
B	Glycerin Plantaren® 818 UP Lamesoft® PO 65 D-Panthenol 75 W Hydagen® Aquaporin Sodium Benzoate	Glycerin Coco-Glucoside Coco-Glucoside, Glyceryl Oleate Panthenol Glyceryl Glucoside, Glycerin Sodium Benzoate	2.00 20.00 1.00 0.50 1.00 1.00	Humectant Surfactant Rheology modifier Active ingredient Active ingredient Preservative
C	Citric Acid (50% solution)	Citric Acid	q.s.	pH Adjustment

Specifications	
pH value (23°C)	4.5-5.3
Viscosity (Brookfield; RVT; spindle TE, Helipath; 10 rpm; 23°C)	60,000-80,000 cP

Jammin' Jelly Handwash HB-US-20-31198-58

Phase	Ingredients	INCI	% by weight	Function
A	Water, demin	Aqua	60.17	
	Cosmedia® Ultragel 300	Polyquaternium-37	4.50	Rheology modifier
	Glycerin	Glycerin	2.00	Humectant
	Plantaren® 818 UP	Coco-Glucoside	25.00	Surfactant
B	Lamesoft® PO 65	Coco-Glucoside, Glyceryl Oleate	1.00	Rheology modifier
	D-Panthenol 75 W	Panthenol	0.50	Active ingredient
	Cibafast® H Liquid	Sodium Benzotriazolyl Butylphenol Sulfonate, Buteth-3, Tributyl Citrate	0.10	Light stabilizer
	Red 40 (0.5% solution) *(Sensient Technologies Corporation)*	CI 16035	0.50	Colorant
	Preservative		1.00	Preservative
C	Fragrance	Parfum	0.50	Fragrance
	Eumulgin® VL 75	Lauryl Glucoside, Polyglyceryl-2 Dipolyhydroxystearate, Glycerin	2.75	Emulsifier (O/W)
D	Hydagen® Aquaporin	Glyceryl Glucoside, Glycerin	1.00	Active ingredient
	Benzalkonium Chloride *(Sigma Aldrich)*	Benzalkonium Chloride	0.13	Antibacterial
	Citric Acid (50% solution)	Citric Acid	0.85	pH Adjustment

Specifications	
pH value (25°C)	5.0-5.5
Viscosity (Brookfield; RVT; spindle 6; 10 rpm; 23°C)	60,000-80,000 cP
Appearance	Transparent liquid

Fresh You" Intimate Cleansing Gel HB-PL-20-16-9

Phase	Ingredients	INCI	% by weight	Function
A	Water, demin.	Aqua	63.80	
B	Dehyton® SFA	Cocamidopropyl Betaine, Disodium 2-Sulfolaurate	9.00	Surfactant
	Dehyton® PK 45	Cocamidopropyl Betaine	18.00	Surfactant
	Lamesoft® PO 65	Coco-Glucoside, Glyceryl Oleate	1.50	Lipid layer enhancer
C	Hydrasensyl Glucan Green	Aqua, Pentylene Glycol, Beta-Glucan, Caprylyl Glycol	3.00	Active ingredient
	Hydagen® Aquaporin Perfume	Glyceryl Glucoside, Glycerin	3.00	Active ingredient
	Lactic Acid	Parfum	0.50	Fragrance
	Sodium Benzoate	Lactic Acid	0.70	pH Adjustment
		Sodium Benzoate	0.50	Preservative

Specifications	
pH value (23°C)	4.50
Viscosity (Brookfield; DV-I+; spindle 2; 10 rpm; 23°C)	1200

Disclaimer

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