

HYDROVEG® – A Moisturising Synergetic Power Blend

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Introduction

The skin plays a fundamental role both as a reservoir and as a barrier to balance the water regulation of the body^{1, 2)}. The water content in the horny layer of the skin may range from a maximum of 20% in the inner and more compact area, to a minimum of 7-10% in the external part. Values superior to 20% can lead to an excessive proliferation of cutaneous microorganisms and cause weakness of the keratin substrates; values inferior to 7% can cause excessive flaking and hyperkeratosis, an abnormal stratification of the epidermis with a consequent increase of skin roughness.

In the stratum corneum different types of substances are present: urea, amino acids, salts and carbohydrates can bind water, all together forming the “Natural Moisturizing Factor” (NMF). Other substances constitute a barrier that limits the transepidermal water loss (TEWL). Among them complex lipids (ceramides) originating from the vital layers of the epidermis play the main role.

Many external factors can influence the water regulation of the skin³⁻⁹⁾. Hydroveg® is a cosmetic ingredient especially made up of a functional association of moisturizing and humectant agents that is particularly recommended whenever an intense and immediate restoring action is necessary. Corneometric tests and innovative studies conducted on biological models of reconstructed skin prove the effectiveness of Hydroveg® in restoring skin to the conditions of physiological balance with a mechanism that is active at the molecular level.

Origin and composition

Hydroveg® is a synergetic blend of moisturizing, humectants and swelling ingredients specifically aimed to correct skin dryness. It was developed by taking into account the normal constituent of a healthy skin, in order to propose a cosmetic raw material having characteristics very similar to human NMF whose composition is evidenced in Table 1.

The NMF is composed by ingredients that beside all, being capable of binding water, can be differentiated for their moisture binding properties at various humidities, providing a synergic activity with

NMF components	%
Amino acids	40.0
Na ⁺ , Ca ²⁺ , K ⁺ , Mg ²⁺ , PO ₄ ²⁻	18.5
PCA (pyrrolidone carboxylic acid)	12.0
Lactates	12.0
Sucrose, organic acids, peptides, others	8.5
Urea	7.0
NH ₃ , uric acid, glucosamine, creatine	1.5
Citrates	0.5

Table 1. Composition of skin NMF

the skin barrier lipids in maintaining the epidermis properly hydrated. So, in developing a reconstructed moisturising factor, it is important to reproduce as close as possible this natural diversity including ingredients that could give enhanced and immediate moisturising effects, stimulate the cellular renewal processes within the skin.

The transport of water and solutes in a highly organized structure such as the skin plays an essential role in maintaining its homeostasis and makes a direct impact on the efficiency of the skin barrier function and, as a consequence, on its appearance. Skin moisturization involves the entire skin structure through active and passive mechanisms (Figure 1). The horny layer acts as a defensive barrier, limiting an excessive evaporation of water (TEWL), while specific channels and the NMF retain the water and transport it through the multi layered skin structure.

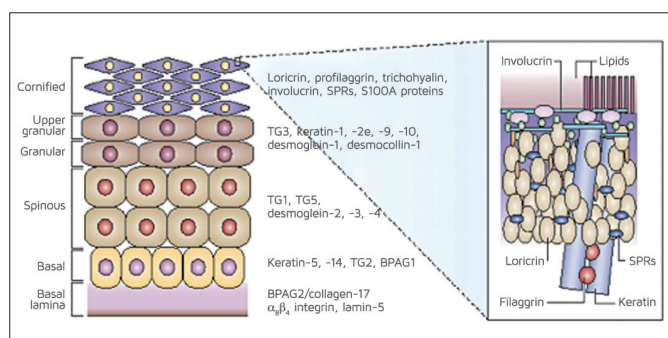


Figure 1. Multilayered skin structure and main epidermal biomarkers.

To meet these specific needs, Variati has developed two versions of Hydroveg® (Table 2). Hydroveg® VV consists of hydrolyzed wheat protein, whereas Hydroveg® R consists of hydrolyzed rice proteins.

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Trade name	INCI	Origin	Applications	Suggested dose
Hydroveg® VV	Aqua, Sodium PCA, Diglycerin, Urea, Hydrolyzed Wheat Protein, Sorbitol, Lysine, PCA, Allantoin, Lactic Acid	Moisturizing complex, designed according to NMF composition	Specifically recommended for soothing & moisturizing body care cosmetics, hand care and massage creams	1 – 5 %
Hydroveg® R	Aqua, Sodium PCA, Diglycerin, Urea, Hydrolyzed Rice Protein, Sorbitol, Lysine, PCA, Allantoin, Lactic Acid	Moisturizing complex, designed according to NMF composition. Gluten free	Specifically recommended for soothing & moisturizing body care cosmetics in allergy prone individuals	1 – 5 %

Table 2. Composition of Hydroveg® VV and R and their use

That is because rice is one of the few plant sources of proteins that can actually be claimed gluten-free. In addition, rice proteins are known for their characteristics of mildness, and this is why they are often included in formulations for sensitive individuals.

The cosmetic properties of Hydroveg® result from the characteristic effects of each single ingredient constituting the product.

Urea^{8,9)}: The particular crystalline structure and the low molecular weight of urea is related to its water binding properties. Urea can increase water retention of keratin by activating the polar groups (SH) of the protein. Furthermore, it possesses desquamating properties. More recent studies have shown the capability of the material to reduce TEWL and diminish the irritative response to sodium lauryl sulphate.

Allantoin¹⁰⁾: The moisturizing properties of allantoin are related to its effect on increasing the capacity of corneocytes to bind water but besides that, allantoin possesses healing properties by promoting cell proliferation and swift epithelialization in skin areas which are injured and stressed following exposure to harsh weather conditions. It also possesses emollient and softening effects on the hardened and hyperkeratosis epidermic tissue and protect the skin by minimizing irritation caused e.g. by surfactants.

Hydrolyzed proteins¹¹⁾: The blend of amino acids obtained from the enzymatic hydrolysis of wheat or rice proteins is very similar to the natural composition of human keratin, respectively. This blend contributes to fix and retain, on a long term, consistent moisture on the skin's surface. Moreover, short chain peptides possess also stimulating properties on the cellular proliferation of skin derived cells.

Lactic acid: A natural alpha hydroxy acid that increases cell renewal processes and the cohesive properties of corneocytes. The carefully selected pH value at which Hydroveg® is formulated minimizes the possibility of irritation.

Sodium PCA, PCA and L-lysine¹²⁾: Pyrrolidone carboxylic acid, stabilized in the form of its sodium salt, from a quantitative point of view is the most important component of NMF. The association of Sodium PCA with PCA and L-lysine, basic amino acid, being less hygroscopic, provides a more balanced moisturizing and hydrating action compared to Sodium PCA alone.

Diglycerin¹³⁾: Diglycerin is a natural moisturizing polyol with plastifying and emollient properties. It reduces the water evaporation and helps to maintain a correct moisture level within the horny layer. Compared to glycerin, it gives longer lasting moisturizing effect and a better skin feel, both properties related to its larger molecular weight.

Sorbitol¹⁴⁾: Hygroscopic molecule with humectant characteristics, able to absorb humidity from the environment.

Skin NMF	Hydroveg®
Amino acids and peptides	Hydrolyzed vegetable proteins
PCA	PCA Na & PCA
Urea and lactates	Urea, allantoin, lactic acid
Sucrose and polyols	Sorbitol and diglycerin

Table 3. Similarities in the composition of Hydroveg® with skin NMF

It can be seen in Table 3 that Hydroveg® composition is very similar to the skin NMF. All ingredients constituting Hydroveg® have a long history of use and on the basis of the available data, they are all considered safe for normal use in cosmetic products¹⁵⁾. This allows Hydroveg® to act intensively and immediately to restore skin that is imbalanced and need action even when the skin is prone to be very sensitive.

How Hydroveg® works

Cellular proliferation and protein assay

Keratinocytes are epidermal cells specialized in the keratin synthesis. Fibroblasts form the site of the synthesis of the extracellular matrix, including collagen and elastin, capable of imparting texture and compactness to the skin. The effect of the product on cellular proliferation and on the increased synthesis of the total proteins on fibroblasts and keratinocytes of skin origin was assessed by in vitro tests. Stimulation of cellular proliferation was monitored by MTT (tetrazole salt) assay at 24, 48 and 72 h and calculated as vitality percentage compared to untreated cells. A maximum increase of cellular proliferation of 9.6% after 48 hours of exposure to the concentration of 2.5 mg/ml Hydroveg® VV was found in the experiment

conducted on fibroblasts. An increase after 48 hours of exposure and at all the tested concentrations, with a maximum of 12.9% at the concentration of 5 mg/ml Hydroveg® VV was found in the experiment conducted on keratinocytes. The protein assay, conducted adopting the Bradford test, showed a protein neosynthesis increase compared to the untreated control both in the fibroblasts (21.8%) and keratinocytes (11.2%), after 48 hours of exposure and at 5 mg/ml Hydroveg® VV.

Test results showed the ability of Hydroveg® VV to stimulate in vitro cellular proliferation and protein synthesis, which suggests a positive effect on cellular turnover and skin renewal and on the ability to support trophism of skin tissue and dermal compactness.

Reconstructed Human Epidermis (RHE) test: skin dryness model

Modern techniques for evaluating the effectiveness on the skin of a cosmetic ingredient are more and more often aimed to stimulate the physiological mechanisms and skin characteristics, carrying out specific in vitro evaluations together with traditional in vivo tests. In vitro skin dryness model uses samples of RHE to recreate in vitro the molecular and physical modifications occurring in dry skin. The test evaluates the ability of a substance to counteract these changes or to replace the tissue homeostasis, lost in the dryness model, monitoring the genic expression of relevant biomarkers, aquaporins, also by morphological analysis and by immuno-localization. The experimental protocol, based on the assessment of the moisturizing capacities in induced skin dryness conditions, made it possible to investigate the activity of Hydroveg® R at the molecular level. The aquaglyceroporine-3 (AQP3) expression has been examined. AQP3 is a protein associated with the membrane, whose role is to transport and distribute water and glycerin and in regulating the cellular skin differentiation. The RHE was stressed for 24 hours by modifying the external environmental conditions with increased temperature and decreased humidity. Hydroveg® R was then applied on dried RHE for 16 hours. The quantification of the moisturizing efficiency of Hydroveg® R was evaluated against glycerin by simultaneously monitoring the localization and expression levels of AQP3. In normal conditions, the expression of AQP3 is preferentially localized in the basal and suprabasal skin layer (Figure 2a).

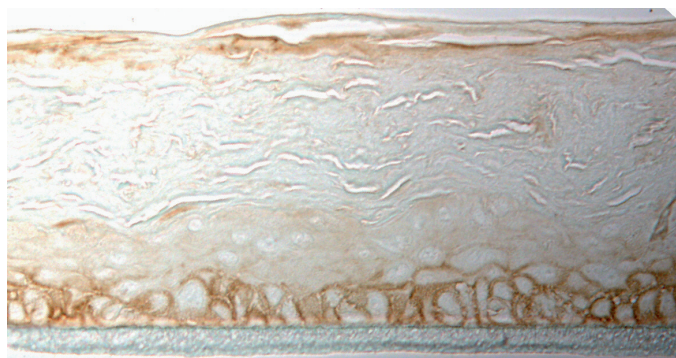


Figure 2a. Immuno-localization of AQP3 in the basal and suprabasal levels in RHE in normal conditions.

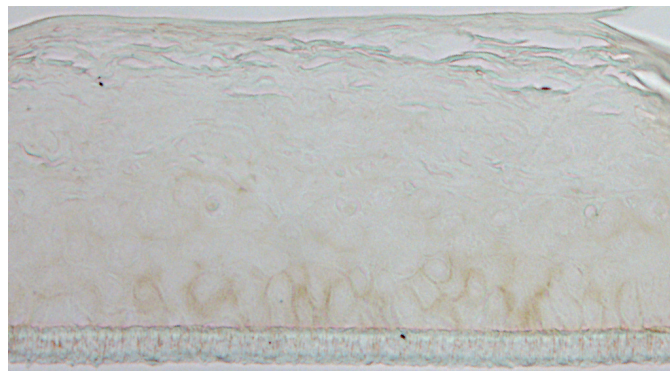


Figure 2b. Immuno-localization of AQP3 - RHE under stress.

Following to the induced skin dryness stress, a dramatic decrease of AQP3 occurs (Figure 2b).

The immuno-histochemical analysis confirmed the over-expression of the protein following treatment with 2.5% Hydroveg® R (Figure 3b), compared both to the “dry skin” control (Figure 2b) and treatment with 5% glycerin (Figure 3a). Additionally, the localization of AQP3 is manifested with a high basal level intensity, as observed in physiological conditions, which suggests a “conservative” mechanism able to bring the skin back to physiological homeostasis conditions (Figures 2a and 3b).



Figure 3a. Immuno-localization of AQP3 - RHE after treatment with 5% glycerin.

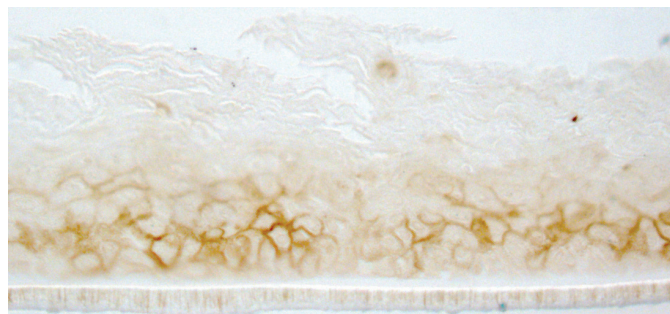


Figure 3b. Immuno-localization of AQP3 - RHE after treatment with 2.5% Hydroveg® R.

The test results showed that the treatment with glycerin does not significantly overexpress the AQP3 gene ($RQ < 2$), while Hydroveg® R significantly increases its expression ($RQ > 2$), which suggests a moisturization mechanism active at the molecular level (Figure 4).

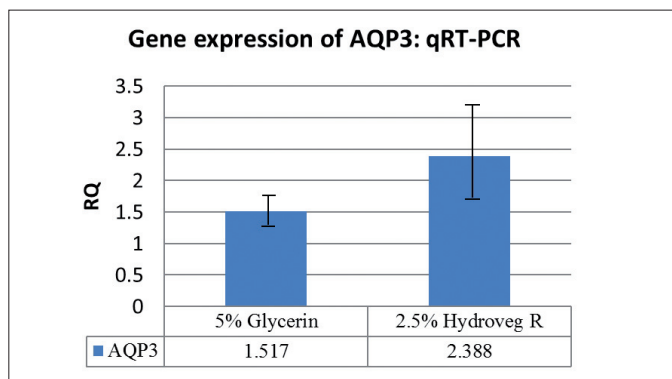


Figure 4. AQP3 gene expression levels.

The test results lead to the conclusion that HYDROVEG® R supports and reinforces the physiological activity of the NMF and gives an immediate response in order to restore the efficiency of the channels that transport water, when it fails, as in dryness conditions.

Clinical tests – Immediate moisturizing power

A corneometric test on 20 subjects, male and female, between 18 and 70 years of age was carried out to determine the moisturizing power of Hydroveg®. The test was conducted using Hydroveg® VV in a carbomer gel with concentrations of 2.5% (Sample A) and 5% (Sample B). Sample A and Sample B were compared against the control sample not containing the active ingredient (Table 4).

Ingredients	Control	Sample A	Sample B
Aqua	qs to 100%	qs to 100%	qs to 100%
Hydroveg® VV	---	2.5%	5.0%
Carbomer	0.7%	0.7%	0.7%
Triethanolamine	0.5%	0.5%	0.7%
Preservatives	0.5%	0.5%	0.5%

Table 4. Tested formulations: without Hydroveg® VV (control), with 2.5% Hydroveg® VV (Sample A) and 5% Hydroveg® VV (Sample B)

The samples were applied in three specific areas of the forearm with a similar moisturization index. The moisturizing difference was assessed by analysing values taken using the corneometer before and

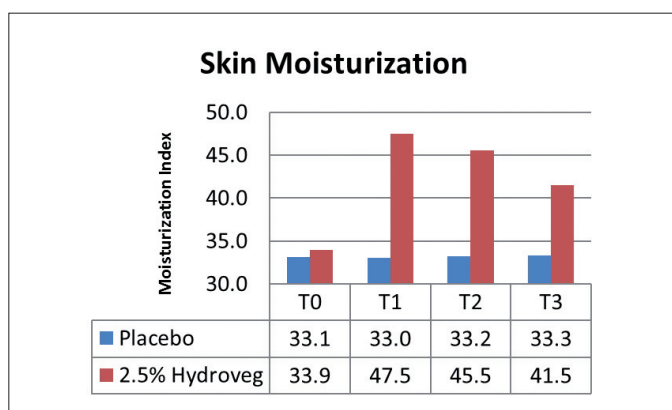


Figure 5a. Moisturizing efficacy of 2.5% Hydroveg® VV.

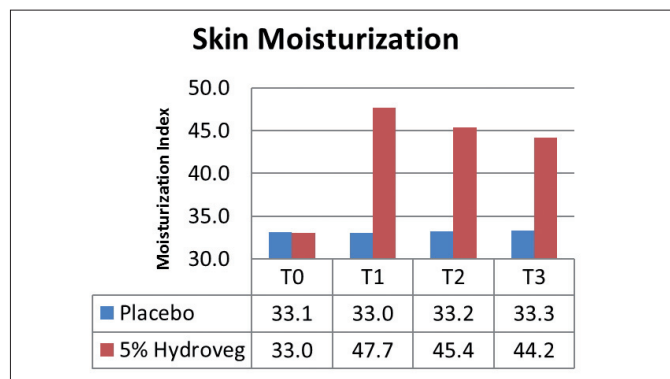


Figure 5b. Moisturizing efficacy of 5.0% Hydroveg® VV.

after the application. The readings were taken at T0 (baseline), 15 minutes (T1), 2 hours (T2) and 4 hours (T3) after applying the product. The statistical analyses show a significant increase ($p < 0.05$) in the moisturizing indices following the application of the tested products compared to the initial value (Figure 5a, 5b). The data confirm that both samples have significant efficacy, suggesting use of the active ingredient in situations where an intense and immediate moisturizing action is needed.

Applications

Hydroveg® gives the cosmetic products a strong and immediate skin moisturizing action and is therefore indicated for making various types of formulation, such as

- Hand and body creams
- Protective day creams
- Firming and massage creams
- Treatment creams for dry and hyperkeratotic skins
- Facial cleansers
- Nourishing/moisturizing hair conditioners

Recommended dosage for Hydroveg® products is 1 - 5%. Example of a body care lotion see in Table 5.

PHASE A	%	PHASE C	%
Aqua (Water)	qs 100	Hydroveg® VV	3.00
Carbomer	0.30	PHASE D	%
Butylene Glycol	3.00	Triethanolamine	0.30
PHASE B	%	Preservatives	as needed
Steareth-21	3.00	Parfum	as needed
Steareth-2	2.00		
Glyceryl Stearate	2.00		
Cetyl Alcohol	2.00		
Caprylic/Capric Triglyceride	5.00		
Isononyl Isononanoate	3.00		
Isostearyl Palmitate	4.00		
IS Avocado	3.00		
Dimethicone	1.00		

Preparation:
Combine ingredients of phase A and heat to 70-75°C. Combine ingredients of phase B and heat to 70-75°C. Add phase B to phase A under stirring. At 30°C, add phase C, preservatives and parfum. Adjust pH between 5.5-6.0 with phase D.

Table 5. Body care lotion containing 3% Hydroveg® VV

Conclusion

Hydroveg® products are perfect designed to meet today's expectation of moisturizers being highly effective and safe but also having high cosmetic acceptance. Another point is that Hydroveg® is easy to use in production since it is a liquid. In vitro tests on cellular cultures and RHE have shown the ability of Hydroveg® to stimulate cellular vitality and renewal, suggesting an overall improvement of appearance, softness and elasticity of skin tissue. Clinical trials show the performance to moisturize the skin significantly and to perform a smooth skin feeling. They have also confirmed the absence of irritating action to the skin and eyes and the absence of allergenic potential. Hydroveg® can be stated to be hypoallergenic (up to 5%) under normal conditions of use (non-irritating and non-allergenic).

References

- 1) Barel A, Paye M, Maibach H. *Handbook of Cosmetic Science and Technology* 2001; 256.
- 2) Blank IH. *Factors which influence the water content of the stratum corneum. J Invest Dermatol* 1952; 18:433-440.
- 3) Siddappa K. *Dry skin conditions, eczema and emollients in their management. Indian J Dermatol Venereol Leprol* 2003; 69(2):69-75.
- 4) Marino Cb. *Skin Physiology, Irritants, Dry Skin and Moisturizers. MPH Report Number 56-2-2001. Washington State Department of Labor and Industries. Safety and Health Assessment and Research for Prevention Program; August 2001.*
- 5) Harding CR, Watkinson A, Rawlings AV, Scott. *Dry skin, moisturization and corneodesmolysis. Int J Cosmet Sci* 2000; 22:21-52.
- 6) Engelke M, Jensen JM, Ekanayake-Mudiyanselage S, Proksch E. *Effects of xerosis and ageing on epidermal proliferation and differentiation. Br J Dermatol* 1997; 137:219-25.
- 7) Jacobi O. *Moisture regulation of the skin. Drug Cosmet Ind* 1959; 84:732-812.
- 8) Loden M. *Urea-containing moisturizers influence barrier properties of normal skin. Arch Dermatol Res* 1996; 288:103-107.
- 9) Loden M. *Barrier recovery and influence of irritant stimuli in skin treated with a moisturizing cream Contac Derm* 1997; 36:256-260.
- 10) Eggensperger H. *Allantoin und seine Derivate, Multiaktive Wirkstoffe für Kosmetika* 1995; 9-33.
- 11) Frei V, Perrier E, Orly I, Huc A, Augustin C, Damour O. *Activation of fibroblast metabolism in dermal and skin equivalent model: a screening test for the activity of peptides. Int J Cosmet Sci.* 1998; 20(3):159-73.
- 12) Zanotti F. *Piroglutamati di arginina e lisina come fattori umettanti. Il prodotto Chimico* 1982; March:25-28.
- 13) Lower E. *The Cosmetic Advantages of Polyglycerols. Manufacturing Chemist* 1997; 68(11):30-32.
- 14) Rovesti P, Ricciardi D. *New experiments on the use of sorbitol in the field of cosmetics. P & EOR* 1959; 771-774.
- 15) *Amended Final Report on the Safety Assessment of Hydrolyzed Rice Bran protein - Int Journal Toxicology March* 2006; 25:91-120. ■

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