

GENENCARE® OSMS

UNLOCK THE POWER OF NATURE

From Nature to Nurture™



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DISCOVER OSMOLYTES: NATURE'S SECRET FOR MOISTURE & PROTECTION

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SUMMARY

A new product line of all-natural organic osmolytes, GENENCARE® OSMS, has been developed by DuPont Industrial Biosciences. It consists of different grades of betaine and recently inositol has been added to this family. When the skin is stressed by external aggressions (UV radiation, heat or cold environments), the water balance of skin cells is disturbed. Osmolytes have evolved in nature to manage skin water balance, reduce water stress and protect the living organism in general.

Betaine and inositol are major osmolytes present in the living layers of the skin. Moreover, betaine and inositol are also important members of NMF (Natural Moisturizing Factor) in the more superficial stratum corneum. This article focuses on betaine and describes how osmolytes contribute to skin protection, not only by protecting cells from dehydration, but also by preventing skin proteins from denaturation.

Various *in vitro* as well as *in vivo* studies are presented, demonstrating the importance of osmolytes in personal care.

INTRODUCTION

Biotechnology represents a discipline that does not regard nature and technology as contradictory but focuses on how they influence each other. For centuries, human beings have been using solutions invented by nature over millions of years as a source of inspiration for technical innovations. Unlocking the power of Nature is the foundation of GENENCARE® OSMS, the osmolyte product line.

When the skin is stressed by external aggressions (UV radiation, heat or cold environments), the water balance of skin cells is disturbed. Nature has developed a magnificent mechanism to manage the water balance, reduce water stress and protect the living organism.

The process by which water management in

“BETAINE AND INOSITOL PLAY AN ESSENTIAL ROLE NOT ONLY TO PROTECT SKIN CELLS FROM OSMOTIC STRESS BUT ALSO TO ASSIST PROTEIN FOLDING AND STABILIZE THE NATIVE STRUCTURE OF PROTEINS”

an organism and in skin are controlled and regulated is called osmosis. Osmosis is the spontaneous net movement of solvent molecules through a semi-permeable membrane into a region of higher solute concentration. Osmosis provides the primary means by which water is transported into and out of cells as response to hypotonic (external medium is less concentrated than inside the cell) or hypertonic (external medium is more concentrated than inside the cell)

Osmosis. Control of water balance in living cells in order to maintain cell volume and preserve intracellular solution for optimal metabolic activities and protect macromolecular structure against osmotic stress.

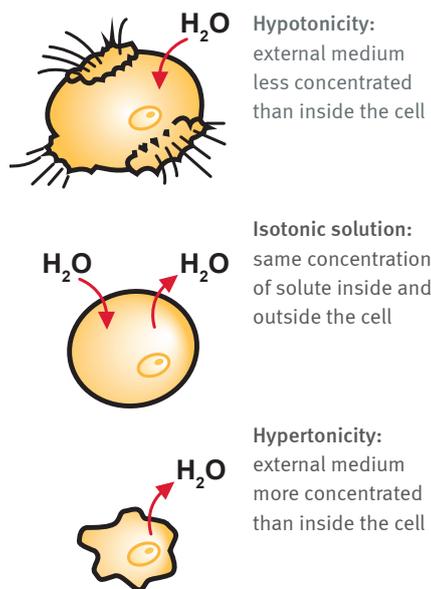


Fig 1. Osmosis

conditions, in order to realize isotonic conditions, where no solute exchange is needed and cells function in an optimal way (Fig. 1). This is realized by the evolution of unique molecules which are able to carry water across cell membranes; these unique molecules are called osmolytes. DuPont Industrial Biosciences has developed a process to extract two major osmolytes, betaine (GENENCARE® OSMS BA) and inositol (GENENCARE® OSMS MI). These are 100 % natural, organic osmolytes, naturally occurring in all living cells, especially in beet root cells, from which they are extracted and purified.

In this article we describe various studies,

demonstrating the important roles of these osmolytes in controlling water balance and protein stabilizing effects as major benefits for new innovative cosmetic products.

Description and Functions of Osmolytes

Osmolytes represent different chemical classes that occur naturally in all living organisms. They consist of amino acids (e.g. proline and glycine), methyl-amines (e.g. betaine and trimethylamine-N-oxide) and polyols (e.g. inositol and sorbitol) [1,2].

Osmolytes fulfil two major roles: (1) Controlling and regulating osmotic processes in order to preserve intracellular conditions for optimal metabolic activities and (2) Protect macromolecular structures against osmotic stress.

Many plants accumulate osmolytes in response to dry or saline conditions, and therefore osmolytes are also often called osmoprotectants [3].

Betaine and inositol are major osmolytes in skin and thus they play an essential role not only to protect skin cells from osmotic stress but also to assist protein folding and stabilize the native structure of proteins [4,5].

Interestingly, both betaine and inositol are also very good moisturizers and their water-binding properties can moisturize and consequently soften and elasticize the upper layers of the skin, i.e. the stratum corneum. However, this benefit is not covered in this article.

Osmolytes and Water Management

Water homeostasis of the epidermis is important for the appearance and physical properties of the skin, as well as for water balance in the body.

Betaine and inositol are organic osmolytes used by keratinocytes to maintain their volume and protect them from external disturbances like hydrous, UV-radiation and thermal stress.

In dry skin, keratinocytes are under hyperosmotic stress. The direct consequence would be a water efflux and

consequent cell shrinkage. Keratinocytes respond by increasing the production of osmolyte transporters on their cell membrane, which results in increased uptake of osmolytes. This increased uptake of osmolytes restores cell hydration and thus normalization of dry skin to normal, well moisturized skin condition.

The main osmolytes functioning in skin are betaine, inositol and taurine and their transporters are betaine/ γ -amino-n-butyric acid (GABA) transporter (BGT1), sodium dependent myo-inositol transporter (SMIT) and taurine transporter (TAUT), respectively. In response to hypertonicity the transport of osmolytes betaine and inositol is increased and accumulate inside the keratinocytes, followed by an osmotic influx of water that restores cell volume [2,6,7].

This is an important regulatory mechanism in skin, such that, in order to maintain water balance and homeostasis of physiological processes, cells can accumulate and release osmolytes like betaine and inositol in response to different external conditions, thus acting as water carriers in or out the cell.

Another important source of an external aggression is oxidative stress caused by UV-A or UV-B radiation. Ultraviolet (UV) exposure induces multiple damaging effects in epidermis and dermis. One of these effects is induction of cell shrinkage due to an opening of K⁺ channels. Thus, it seems that UV-A and UV-B radiation, similar to hyperosmotic stress, causes keratinocyte shrinkage and the subsequent increased uptake of osmolytes like betaine and inositol via increased induction of BGT1 and SMIT transporters, respectively [8,9,10].

Therefore, betaine and inositol are important epidermal osmolytes, required to maintain keratinocyte hydration in a dry or UV-stresses environment.

Osmolytes and Stabilization of Proteins and Other Macromolecules

The environmental stress also disturbs the stability of macromolecular, e.g. protein, configuration and as a consequence various

Osmolytes push the folding equilibrium toward natively folded conformations. The protecting osmolytes raise the free-energy of the unfolded state, favoring the folded population (source: Ref. [4])

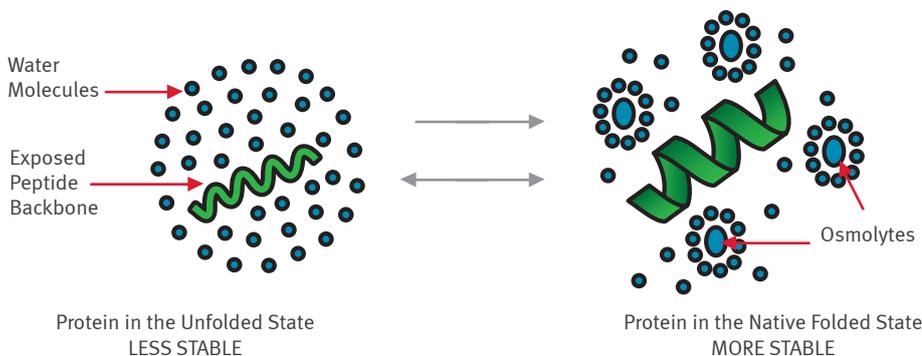


Fig. 2 Protein stabilizing effect of osmolytes

osmolytes have been selected to stabilize the intracellular macromolecules.

It has been shown that the protein backbone is effectively osmophobic, and osmolytes can provide significant stability to proteins by an indirect mechanism. Osmolytes are removing water from the protein first solvation shell, allowing the protein to fold and hide its backbone. The ability of osmolytes such as betaine and inositol to increase the driving force for protein folding is due to a solvophobic effect on the peptide backbone exposed in the unfolded state. Another feature of these molecules is that they make the unfolded state of macromolecules such as proteins in osmolyte solution very unfavorable relative to the folded state, without changing the rules of protein folding that occur in dilute solutions [4,11].

Organic osmolytes are used widely in nature to protect cellular proteins against harsh conditions such as the effects of dehydration, other hypertonic states, or the buildup of potentially denaturing metabolites. Hyper-osmotic conditions set off a response in most cell types that turns on the synthesis of protective organic osmolytes. Therefore, a thermodynamic process of protein folding can be framed in terms of solvent interactions with the unfolded and native states. Osmolytes act by increasing the Gibbs free energy (ΔG) between the natively folded and unfolded structures, thus pushing the folding equilibrium toward natively folded conformations [12].

Thus, osmolytes, by attracting water,

increase thermodynamic stability of the native folded state of the proteins, but without direct interaction with the protein, without interfering with cellular processes and without perturbing cellular structures (Fig. 2).

DESCRIPTION OF GENENCARE® OSMS BA

GENENCARE® OSMS BA is betaine extracted from non-GMO sugar beets (*Beta vulgaris*), represents a renewable raw material source, is naturally sourced and from plant origin. (Fig. 3). GENENCARE® OSMS BA is a trimethylated amino acid derivative, also referred to as

Both betaine and inositol are extracted from sugar beets, thus 100 % naturally sourced and 100 % plant origin.

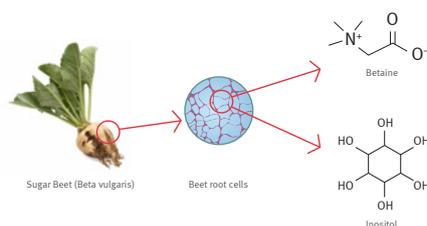


Fig. 3 Sourcing of betaine (GENENCARE® OSMS BA) and inositol (GENENCARE® OSMS MI)

Betaine acts as water-carrier: it attracts water but it does not immobilize it. (Source: Ref.[14])

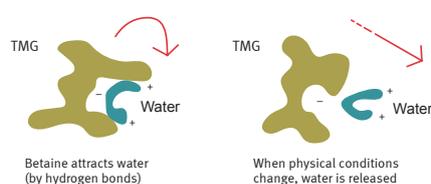


Fig. 4 Water binding of betaine

trimethylglycine or glycinebetaine or TMG; the INCI-name is Betaine.

Betaine is a small molecule (molecular weight of 117 daltons) which strongly interacts with water and polar molecules. It is both an amphoteric (i.e. can react as an acid as well as a base) and an amphipathic (i.e. it has a hydrophobic and a hydrophilic group) molecule. At its isoelectric point, betaine exists in the zwitterionic form, i.e. both negatively and positively charged.

Since GENENCARE® OSMS BA is 100% naturally sourced and 100% plant origin, it has Ecocert, Cosmos and Natrue certifications.

In aqueous solutions betaine forms strong hydrogen bonds, thus changing the water activity. The bound water, however, is not immobilized, but there is easy exchange of water molecules between the hydration sphere of betaine and the bulk water. In other words, one water molecule, when attracted by a betaine molecule, is very rapidly substituted by another water molecule. That is, it has a shorter residence time in the vicinity of betaine than in that of another water molecule. Betaine is therefore a true water carrier that releases it easily to the surrounding environment when required by changing physical conditions [13,14] (Fig. 4).

It is important to note that GENENCARE® OSMS BA is not the surfactant cocoamidopropylbetaine or other betaine-surfactants, which are often also called betaine in short as abbreviation. These are surfactants which are manufactured by chemical synthesis and have very different functionalities, e.g. detergency, in personal care products.

DESCRIPTION OF GENENCARE® OSMS MI

GENENCARE® OSMS MI is inositol and, like GENENCARE® OSMS BA, extracted from sugar beets (*Beta vulgaris*) (Fig. 3). Inositol exists in 9 stereoisomers: L- and D-chiro, myo-, neo-, scyllo-, muco-, cis-, epi- and allo-inositol, of which the most prominent form, widely occurring in nature, is myo-inositol.

GENENCARE® OSMS MI is myo-

inositol, and will be referred to as inositol in this article.

Also like betaine, inositol GENENCARE® OSMS MI is 100 % naturally sourced and 100% plant origin. Inositol was considered a member of the vitamin B complex (formerly Vitamin B8); however, because it is produced by the human body from glucose, it is officially not a vitamin, but rather an essential nutrient, since it can be synthesized in the body (including skin), but is not made in amounts considered adequate for good health.

Inositol fulfills many biological activities in skin. As mentioned above, it is one of the major skin osmolytes, regulating water-management and cellular volume as well as enhancing protein-stability. Moreover, as a polyol, it is a very good moisturizer [15].

Next to these important functions, it is the constituent of phosphatidyl-inositol (PI) and as such is involved in the phosphoinositide cascade: hydrolysis by a phospholipase C results in the formation of diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP3), both having important communication functions as mediators of intracellular signal transduction and external information [16,17,18,19]. IP3 is an important second messenger and involved in Ca²⁺ mediated signals, by opening Ca²⁺ channels in plasma membranes to release and raise intra-cellular, cytosolic Ca²⁺ levels, which regulates proliferation and differentiation of e.g. keratinocytes [20].

BENEFITS AND APPLICATIONS OF OSMOLYTES

The major benefits of osmolytes for personal care in general, and betaine in particular, are:

■ CONTROL OF SKIN WATER BALANCE

Skin cells (keratinocytes) are actively using organic osmolytes to carry water inside or outside cells in order to maintain homeostasis and protect skin from dehydration on a cellular level.

■ STABILIZATION OF SKIN MACROMOLECULES IN GENERAL AND SKIN PROTEINS IN PARTICULAR

This is not only protecting proteins from denaturation, but also enhancing the folding of proteins into correct secondary and tertiary configurations.

■ IMMEDIATE SKIN MOISTURIZATION

Osmolytes, especially betaine and inositol, are strong humectants. They are wellknown components of Natural Moisturizing Factor (NMF).

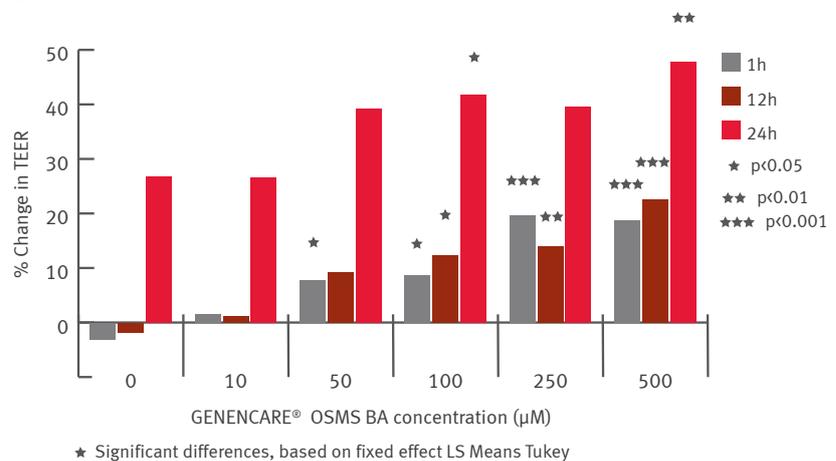
■ LONG TERM SKIN MOISTURIZATION

By controlling water balance and stabilizing proteins, osmolytes play important roles in cells cohesion and skin barrier formation (see above and also below under Substantiation)

■ LONG TERM REINFORCEMENT OF SKIN'S BARRIER

This is the result of the above functionalities. It must be realized that well moisturized skin (short term, long term as well as maintaining proper

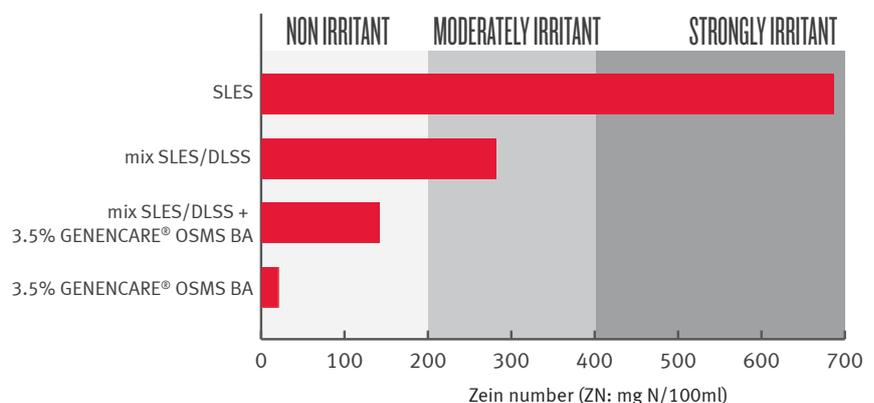
Differentiated keratinocytes were treated with 0-500 µM of GENENCARE® OSMS BA and the strength of tight junctions (TJ) was measured using chopstick electrodes at different time points.



TEER: Trans Epithelial Electrical Resistance
% Change in TEER = Percentage change in TEER calculated from time point 0h; mean are shown

Fig 5. The effect of GENENCARE® OSMS BA on the TEER of differentiated keratinocytes

The Zein test determines the extent of denaturation of a protein derived from corn, which is insoluble in water, by surfactants. Zein protein is incubated with a 1% surfactant solution at 30 °C for 1 hour and the amount of organic nitrogen is determined by the Kjeldahl method. The zein number (ZN) obtained corresponds to the amount of nitrogen (in mg) detected in 100 ml of surfactant solution.



SLES: sodium lauryl ether sulfate; DLSS: disodium laurylsulfosuccinate; mix SLES/DLSS = 50% SLES / 50% DLSS solution

Fig 6. GENENCARE® protective effect on Zein proteins towards surfactants in Zein test.

cell volume) creates conditions under which optimal barrier formation is realized. A well-moisturized skin is able to have (a) proper keratinocyte differentiation into corneocytes, (b) proper lipid biosynthesis and (c) proper secretion of these lipids (mainly ceramides, free fatty acids and cholesterol (esters) from lamellar bodies at the stratum granulosum and stratum corneum interface into the intercellular spaces, where they form multiple bilayers and thus forming, together with the corneocytes, the 'brick (= corneocytes) and mortar (= lipids)' barrier-structure [21,22,23].

■ **HAIR STRENGTHENING**

Osmolytes can also be used beneficially in hair care by strengthening hair and protecting it from heat damages. This is the consequence of the even penetration of betaine into the hair shaft and consecutive increase of water retention.

In the next section, a number of studies (*in vitro*, *ex vivo* as well as *in vivo*) are presented, which have been performed to substantiate these benefits.

SUBSTANTIATION

The focus of the studies presented here is on the protein stabilizing and barrier strengthening effects of betaine. Short and long term moisturization effects of GENENCARE® OSMS BA as a humectant and as an osmolyte are published and described elsewhere. Studies with GENENCARE® OSMS MI are ongoing, since it is a relatively new member of the GENENCARE® OSMS family. The long term *in vivo* application test (see below) shows the benefits of a blend of betaine and inositol.

IN VITRO STUDIES

Stabilizing effect of betaine on tight junctions (TJ)

Tight junctions are cell-cell junctions, which play a central role in sealing the intercellular space of keratinocytes in

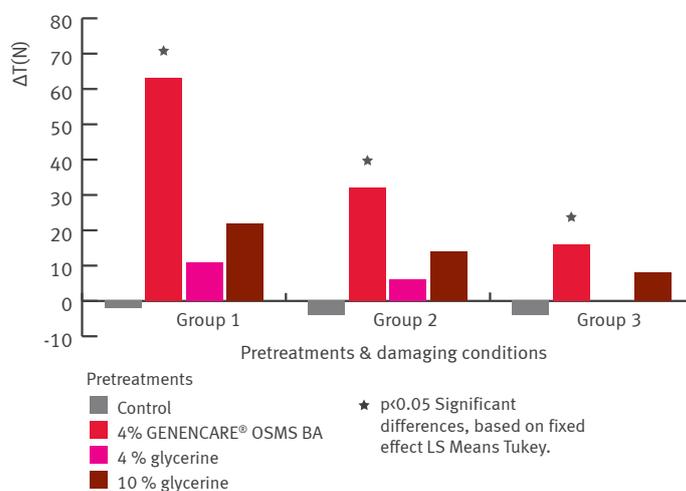
particular at the stratum granulosum level, thereby contributing to barrier function of the skin. Tight junctions (TJ) contribute to the inside-out and to the outside-in skin barrier and are important for preventing water loss [24,25,26,27].

To assess the effect of GENENCARE®

OSMS BA, differentiated keratinocytes were treated with 0-500 μMol of betaine and the strength of TJ was measured using chopstick electrodes at different time points.

During the differentiation the cells form tight junctions between the cells. The

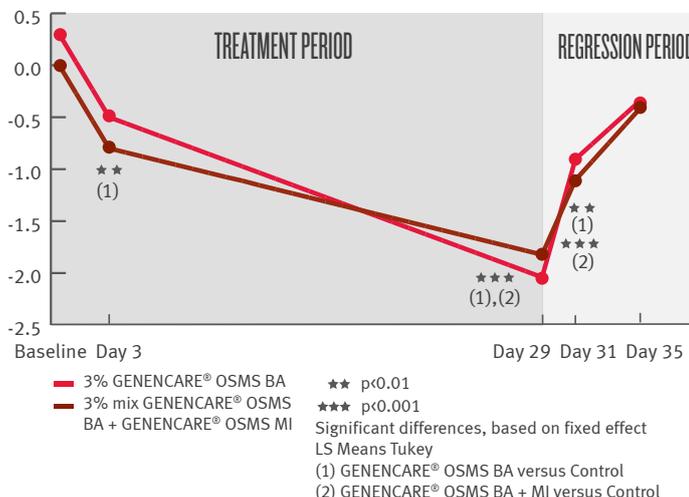
Hair was preconditioned for 24 hours at 32°C and 65% Relative Humidity (RH). After preconditioning, the hair was treated with a commercial shampoo as a control and the same shampoo containing 4% betaine or 4% an 10% glycerol, respectively. Hair was then damaged under three different conditions and tensile strength (T) measurements were performed by monitoring the force (T) that must be applied to reach 0.5% hair elongation (elastic region)



Group 1: 4 hours of drying at 32°C under nitrogen; Group 2: 2 hours of drying at 45°C and 52% RH; Group 3: 4 hours of drying at 45°C and 52% RH.

Fig. 7. The effect of GENENCARE® OSMS BA on tensile strength of Caucasian hair

An O/W polymeric emulsion (placebo, with 3% GENENCARE® OSMS BA and with a blend of 3% GENENCARE® OSMS BA / GENENCARE® OSMS MI) was applied twice a day ad libitum for 29 days. After this period, application was stopped and the TransEpidermal Water Loss (TEWL) was further monitored. n = 41 (20-60 years)



ΔTEWL: difference in TransEpidermal Water Loss compared to control (placebo). Control as baseline (O/W polymeric emulsion)

Fig. 8. Four week application study with GENENCARE® OSMS BA and GENENCARE® OSMS MI blend

cell layer in itself is impermeable to water and water-soluble substances, but the flow of solutes through TJ can be measured by transepithelial electrical resistance (TEER). A greater resistance means stronger TJ between the cells (Fig. 5).

The results show that betaine increases the strength of TJ in a dose dependent manner compared to control without betaine at 24h after application. Already after 1 h this increase can be measured at higher concentrations of betaine. At concentrations > 50 µM, these effects become statistically significant. Therefore, GENENCARE® OSMS BA improves tight junction integrity and thus contributes to the barrier and hydration status of the skin.

Protein stabilizing effect of betaine in Zein test

Zein is a protein derived from corn and insoluble in water. A surfactant solution causes a part of zein to dissolve. There is a very good correlation between the *in vivo* irritation potential of the surfactant

and its ability to denature and dissolve the zein protein, according to reference Götte, 1964 [28,29,30]. In this procedure, zein protein is incubated with a 1 % surfactant at 30 °C for 1 h and the amount of organic nitrogen is determined by the Kjeldahl method. The zein number (ZN) obtained corresponds to the amount of nitrogen (in mg) detected in 100 ml of surfactant solution. The higher the zein number, the higher the *in vivo*-irritation potential of the tested material. Sodium lauryl ether sulfate (SLES) alone is strongly irritating in this test, while a 50 %/50 % mix of SLES with disodium lauryl sulfocuccinate (DLSS) is moderately irritant. The addition of 3.5 % GENENCARE® OSMS BA to this mixed surfactant solution decreases the zein number to such an extent that it becomes non-irritant; this demonstrates a strong reduction of harshness of cleansing surfactants towards proteins, which is indicative for a reduction of their irritation potential *in vivo* (Fig. 6).

EX VIVO STUDIES

GENENCARE® OSMS BA strengthens hair and protects against heat damage

Tensile strength measurements were performed with hair, treated with a commercial shampoo containing 4 % GENENCARE® OSMS BA and compared with hair, treated with the same shampoo without betaine (control) and containing 4 % or 10 % glycerol, respectively. Hair was then damaged under three different conditions: 4 h of drying at 32 °C under nitrogen, 2 h of drying at 45 °C and 52 % RH and 4 h of drying at 45 °C and 52 % RH. Tensile strength measurements were performed by monitoring the force that must be applied to reach 0.5 % hair elongation (in the elastic region) of the standard Force-Elongation curve for human hair according to the method of Mercelot, 1998 [31]. The results (Fig. 7) clearly show that hair, damaged under all three different conditions, but pretreated with 4 % GENENCARE® OSMS BA, is significantly more resistant to elongation and therefore also to breakage. GENENCARE® OSMS BA performs much better than glycerol in this respect

(Fig. 7).

IN VIVO STUDIES

Barrier strengthening effect of GENENCARE® OSMS BA and GENENCARE® OSMS MI in a four-week clinical study.

A clinical study was performed using an O/W polymeric emulsion as placebo and compared with the same emulsion to which 3% GENENCARE® OSMS BA and 3 % of a blend of GENENCARE® OSMS BA/GENENCARE® OSMS MI was added. After 3 days of application (twice a day), both formulations showed a clear reduction in trans epidermal water loss (TEWL) as compared with the placebo and after 4 weeks (29 days) of treatment the TEWL was further reduced and the effects were significant for both GENENCARE® OSMS BA and GENENCARE® OSMS BA/GENENCARE® OSMS MI formulations (Fig. 8). Two days after the treatment period had stopped, the TEWL was still significantly reduced; at day 35, TEWL was also still reduced, but this was not statistically significant. This demonstrates a prolonged effect of GENENCARE® OSMS BA and GENENCARE® OSMS BA/GENENCARE® OSMS MI on the skin barrier integrity. In general, these results show that GENENCARE® OSMS BA and GENENCARE® OSMS MI have complementary benefits.

It is expected that these barrier strengthening effects of GENENCARE® OSMS BA and GENENCARE® OSMS MI are the result of the various efficacies of these unique compounds; not only regulating water volume as osmolytes on a cellular level, but also their protein-stabilizing effects (see above, under tight junctions) as well as their intrinsic moisturizing capacities.

Reduction of irritation potential of surfactants by betaine

A human patch-test was performed, using 10% sodium lauryl ether sulfate (SLES) as a positive control for irritation, and the results were compared using the same SLES solution, with 2%, 3.5%, 5% and 7% GENENCARE® OSMS BA. Fig. 9

Materials tested were 10 % sodium lauryl ether sulfate solution with respectively 0 %, 2 %, 3.5 %, 5 % and 7 % GENENCARE® OSMS BA. Test materials were applied to human volar forearms under occlusion for 24 and 48 hours. Patches were then removed and the skin reaction was assessed by clinical observation of irritation score (scale 0-5). n=25 (20 -60 years).

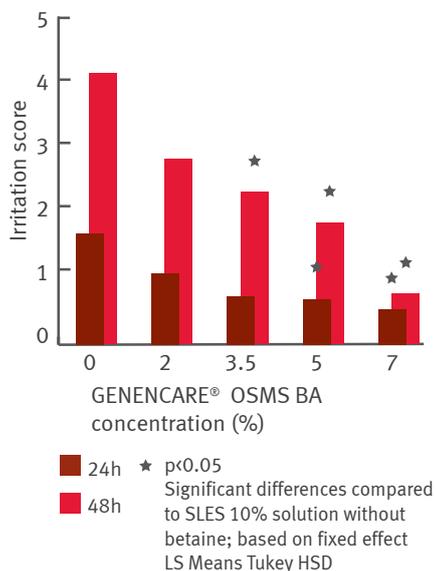


Fig. 9 The effect of GENENCARE® OSMS BA on skin irritation caused by SLES 34

clearly shows that at a level of 3.5% betaine and above, a significant reduction in the irritation by SLES was observed. This effect was dose-dependent and increased when increasing concentrations of GENENCARE® OSMS BA were used.

This test demonstrates that GENENCARE® OSMS BA is able to significantly reduce the irritation potential of surfactants on human skin.

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