03/21 | Volume 147 | Thannhausen, Germany, March 17, 2021

sofw journal

Home & Personal Care Ingredients & Formulations

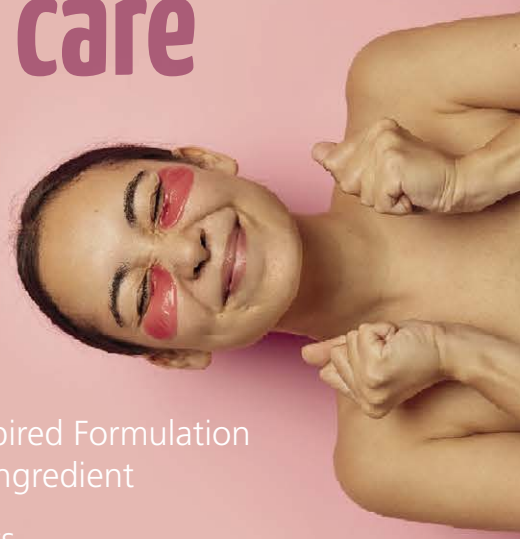
powered by SOFW

personal care

Thickening of Surfactant Formulations Using Worm-like Micelles

Lactobacilli based on True Biotic Technology

03
2021
english



skin care

A Japanese Zen Aesthetic-inspired Formulation with a Root-powered Active Ingredient

New Active Ingredient Protects Skin and Scalp against Silent Inflammation

home care

Effects of Additives on the Phase Behavior of an EO/PO-Copolymer

anti-aging

Key Player: Protein Balance

Active Beauty
B-Lightyl™
The universal magic eraser for dark spots

The absolute sea treasure to even out all skin complexion types

- Take back control on hyperpigmentation disorders
- Reduce the number of visible spots
- Protect against solar & senile lentigo
- Crafted by marine biotechnology

To find out more, contact us at global.cosmetic@givaudan.com

Givaudan

engage your senses

peptides 22
peptides
New Active Ingredient
Unleashed by the Power of AI

environment 40
environment
The Ecological Baggage
of a White Cotton T-shirt in Germany

personal care

home care

Environment

Distillation

Interviews 46-47

Formulations 50-51

Company News 52-55

Thickening of Surfactant Formulations Using Worm-like Micelles

J. Kleinen, J. Venzmer

abstract

A frequently used method to obtain the desired flow behavior of surfactant formulations is to utilize the surfactant aggregates themselves. This can be done by converting spherical micelles to worm-like micelles by addition of a hydrophobic thickener; additionally, the surfactant aggregates can be bridged by using hydrophilic, associative thickeners. In order to understand why some formulations are hard to thicken, the origin of the salt and/or pH curves, or the temperature dependence of viscosity, one should take a closer look at the morphologies and properties of the surfactant aggregates formed.

Introduction

One option to provide formulations with the desired viscosity would be to use (bio-)polymers to just thicken the water phase. However, a more preferred flow behavior can only be obtained by utilizing the surfactant aggregates themselves for building viscosity. A schematic representation typically used to explain thickening of surfactant systems using either hydrophilic (i.e. associative) or hydrophobic thickeners is shown in **Fig. 1** [1]. Adding hydrophobic thickeners to a surfactant system of spherical micelles can cause the transition to worm-like micelles, whereas hydrophilic (a.k.a. associative) thickeners are able to connect surfactant aggregates; more efficient than bridging of spherical micelles to build viscosity would be connecting of worm-like micelles. This is why often a combination of hydrophobic and hydrophilic thickeners is being used.

In order to understand why some formulations are easier to thicken than others, the origin of the temperature-dependence of viscosity, and the phenomena related to the so-called salt curve or the pH dependence of viscosity, we need to take a closer look at those worm-like micelles. Sometimes, also the term rod-like micelles is being used; this indicates already that there are more parameters than just the length needed to describe the behavior of such micelles. In the following, it will be discussed how formulations containing worm-like micelles react to shear forces. All changes to the system which influence length, shape or stiffness of the micelles will have an effect on the flow behavior.

Aggregation of surfactants

The type of surfactant aggregates formed can be understood by simple geometric considerations originally put forward by Israelachvili [2], who introduced the critical packing parameter (CPP) concept. The CPP is defined as $V/a_0 l$, i.e. the ratio between volume of the hydrophobic portion of a surfactant (V) and the area of its hydrophilic headgroup within the interface (a_0) multiplied with the length (l) of the hydrophobic tail (**Fig. 2A**). If the CPP was $<1/3$ (i.e. the surfactant molecule was shaped like an ice cream cone), the surfactant will form – for geometric reasons – a spherical micelle. This is typically the case for single C12 alkyl chain surfactants with a "normal", charged headgroup (e.g. SLES (Sodium Laureth Sulfate) or CAPB (Cocamidopropyl Betaine)). It should be noted that the size of a surfactant's headgroup is more than its bare molecular structure – it includes the hydration sphere, which is mainly influenced by the headgroup charge. Therefore, the headgroup size (and hence CPP) is influenced by e.g. salt level in the formulation as well as interaction between oppositely charged headgroups (for ionic surfactants) or temperature (for ethoxylates). In case of one headgroup was connected to two C18 alkyl chains (e.g. phospholipids, fabric softener quats), the CPP is ≈1 and the surfactants form planar (curvature ≈ 0) bilayers (i.e. vesicles, lamellar phase) (**Fig. 2B**).

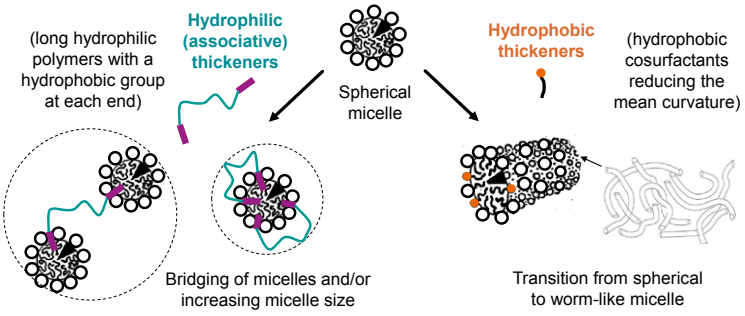


Fig. 1 Thickening of surfactant systems using hydrophilic and hydrophobic thickeners [1].

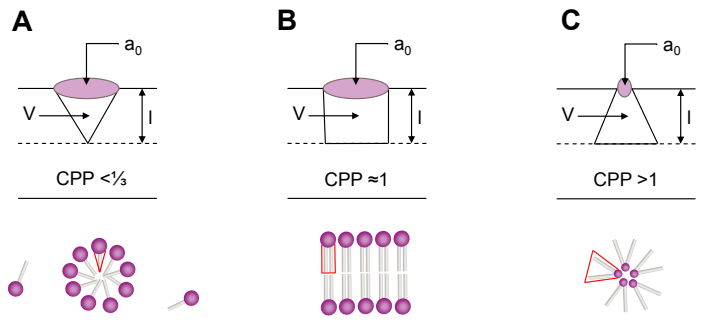


Fig. 2 Packing parameter (CPP) and aggregation of surfactants: A) Single chain surfactants forming spherical micelles; B) Double-chain surfactants forming planar bilayers; C) More hydrophobic surfactants forming inverse micelles [2].

Typical length scales of micelles

The diameter of a spherical micelle – typically few nanometers (nm) – is twice the length of a surfactant molecule, including the hydration sphere of the surfactant's headgroup (**Fig. 3A**). Increasing the packing parameter (i.e. reducing the curvature) can be done by adding less hydrophilic surfactant molecules having only a very small headgroup – typically known as hydrophobic thickeners (a_0 very small, and therefore CPP >1). If the amount of hydrophobic thickener was sufficient, there is a transition from spherical to worm-like micelles.

Looking at the cross-section of a worm-like micelle, there are two options: perpendicular to the long axis, both curvature and diameter are the same as in a spherical micelle (**Fig. 3A**), but along the long axis the curvature is zero, except for the end-cap (**Fig. 3B**). This is ideal to accommodate the hydrophobic thickener molecules. In addition to the thickness of a worm-like micelle, there is the overall end-to-end length named contour length l_c; this can be up to several hundred nm or even few µm. The length of a straight segment of a micelle is called persistence length l_p (**Fig. 3C**). If l_p was in the same range as l_c, one is speaking of rod-like micelles, whereas typical of a worm-like micelle is $l_p << l_c$. But there is even more, in case both length and concentration are sufficiently high to allow overlaps and entanglements to occur: Mesh size ξ and the entanglement length l_e, the average loop length between two knots (= physical crosslinking points) (**Fig. 3D**).

How do we know all this? The most direct method to observe such wormlike micelles is Cryo-TEM [3]; however, this method is technically quite challenging and requires lots of experience, since it is not easy to avoid artifacts. All properties of the micelles in terms of e.g. length, stiffness, entanglements, or branching have an effect on the flow behavior. Therefore, rheological characterization can also provide insight into dimensions, flexibility and interactions of the micelles [4]; this is especially relevant since already subtle changes in surfactant composition, pH, electrolyte and temperature can lead to significant changes in macroscopic flow behavior. In the following, it will be discussed what could or will happen if we want to further increase viscosity by adding more hydrophobic thickener or if we vary the temperature.

A lot does not always help a lot – Salt curve

Building viscosity in a surfactant formulation can be done – as mentioned above – by reducing the mean headgroup size (i.e. increasing the CPP) by adding salt and/or hydrophobic thickener. The easiest example of a model body wash formulation is 9% SLES/3% CAPB/2% NaCl/pH 5.5, which has already a sufficient viscosity – without any further addition of hydrophobic or associative thickeners. At the typical formulation pH of 5.5, a small fraction of the CAPB is protonated (isoelectric point pH 6.25), i.e. interacts with SLES by Coulomb attraction and thereby eliminating each others hydrophilicity. In combination with some salt (reducing the headgroup size of the surfactants), this is sufficient to build worm-like micelles. This has been discussed in detail in [5].

Attempts to further increase viscosity by adding more hydrophobic thickener and/or salt are successful only up to a certain point: Instead of a further increase in the length of the worm-like micelles, there is a formation of Y-junctions or branching points [6]. This is schematically shown in the so-called salt curve (**Fig. 4**). The origin of this branching is shown

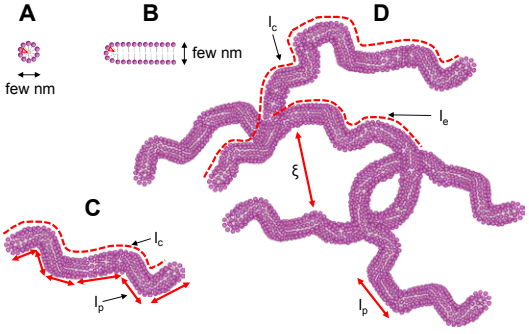


Fig. 3 Typical length scales of micelles: A) Diameter of a spherical micelle; B) Cross-section of a worm-like micelle; C) Contour length lc and persistence length lp of a worm-like micelle; D) Mesh size ξ and entanglement length le in a concentrated solution of worm-like micelles.

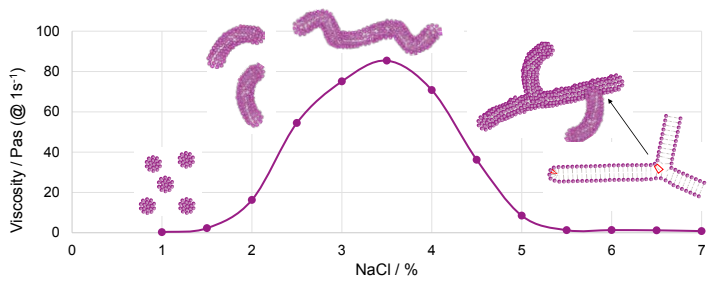


Fig. 4 Salt curve of a simple model formulation (9% SLES/3% CAPB/pH 5.2/x% NaCl/25°C) and a schematic representation of the corresponding changes to the micellar morphology.

in the cross-section: The local curvature at the Y-junction is similar to that of an inverse micelle, which is the curvature favoured by hydrophobic thickeners (CPP>1). One consequence of the formation of branches is a drop in viscosity, because a branched aggregate has a smaller hydrodynamic volume and hence less entanglements with the neighboring micelles, compared to the linear aggregate consisting of the same number of surfactant molecules. Additionally, the formulation might turn turbid, which is an indication for phase separation into a surfactant-rich phase of branched, interconnected micelles dispersed in a surfactant-poor phase.

Similarities and differences of worm-like micelles and polymer chains

From the standpoint of physics, there should be some kind of analogy between worm-like micelles and polymer chains dissolved in a good solvent. De Gennes has described the movement of polymer chains with the reptation theory [7]: The snake-like motion of an entangled polymer chain is explained by imagining that it is confined to a "tube" formed by adjacent chains; the reptation time t_{rep} is defined as the time needed for the chain to completely move out of that tube. However, long worm-like micelles exhibit a much higher fluidity which can not be accounted for by the reptation model. So there must be a general difference between a worm-like micelle and a polymer chain. A worm-like micelle is not a covalently bound chain, but it experiences scission, i.e. breaking of the micelles into segments (characteristic timescale t_{br}) followed by recombination of the broken segments [4]. Since micelles are in thermodynamic equilibrium with single surfactant monomers, there is a permanent exchange of material; the higher the monomeric solubility (i.e. Critical Micelle Concentration, CMC), the more pronounced this exchange is. Therefore, worm-like micelles have two possibilities to release an applied stress: Reptation or reversible scission, depending on the magnitudes of t_{rep} and t_{br}.

Viscoelastic fluids – Maxwell model

Most AT laboratories have (e.g. Brookfield) viscometers to measure the viscosity of formulations, but rarely rheometers to perform oscillatory rheology measurements in order to characterize viscoelastic fluids by determining e.g. storage modulus G' and loss modulus G". A detailed discussion of the difference between viscosity measurements and oscillatory rheology as well as scope and limitations of the two methods is beyond the scope of this paper and will be reported separately.

Let us consider a situation in which A) reversible scission is dominating ($t_{br} << t_{rep}$) and B) the worm-like aggregates are in the semi-diluted regime (i.e. surfactant concentration between overlap concentration of the micelles and a concentration at which the network mesh size ξ is longer than persistence length l_p). In this special case, worm-like micelles show so-called viscoelastic response to applied shear, i.e. a rheological behavior which can be described by the Maxwell model with one single relaxation time $t_R = (t_{br} \times t_{rep})^{½}$ ($\sim\omega_c^{-1}$) (**Fig. 5**).

At low frequencies (= long times scales, at rest), the loss modulus G" (= "viscosity") is larger than the storage modulus G' (= "elasticity"), which means the system (more or less slowly) flows. Here, the timescales are long enough for the worm-like micelles to disengage from their transient network as a reaction to applied stress – the reversible scission is sufficiently fast. At this long timescale, the response of the surfactant formulation is independent to both applied stress and shear; the viscosity is thus constant, and this region translates to the Newtonian plateau value of the viscosity (e.g. **Fig. 8A** (left part of the curve)). At shorter timescales (i.e. frequencies higher than ω_c), the storage modulus G' is dominating, i.e. the rheology reflects an intact, elastic network of worm-like micelles, connected by entanglements. In response to the applied stress, the worm-like micelles align in the direction of flow, and hence the viscosity is decreasing (e.g. **Fig. 8A** (right part of the curve)). The cross-over frequency ω_c corresponds to the so-called structural relaxation time, which is greatly influenced by the alkyl chain length (distribution) of the surfactants (see below). The plateau value of G' at high frequencies is related to the crosslink density or micellar mesh size ξ. In short, a formulation containing worm-like micelles can be characterized by determining G', G" and ω_c.

Temperature dependence of viscosity

The temperature dependence of the viscosity of surfactant formulations is of practical relevance, because the so-called "sportsbag" effect should be avoided: A body wash or shampoo should neither be thick/almost solid in winter, nor thin fluid in summer. Therefore, it is often necessary to study and adjust the viscosity not only at room temperature, but also at 5° and 40°C. This is shown for the above used model formulation based on 9% SLES/3% CAPB/0.7% NaCl/pH 5.2 (**Fig. 6**). The viscosity of this baseline system is quite low – typical of spherical micelles (**Fig. 6A**). Only upon addition of

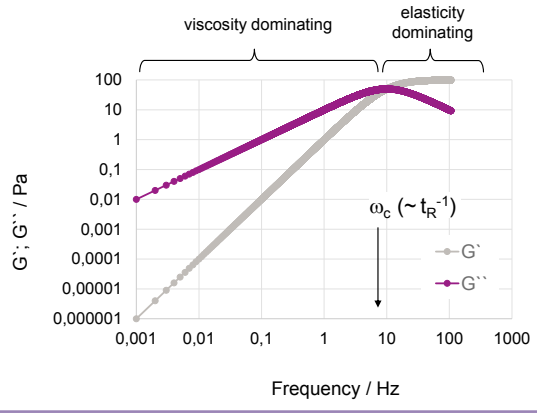


Fig. 5 Flow behavior of a viscoelastic fluid as described by the Maxwell model: G' = Storage modulus (= "elasticity"); G" = Loss modulus (= "viscosity").

more NaCl or hydrophobic thickener (**Fig. 6B/C**), the viscosity at room temperature is in the desired range. The viscosity can be further increased – at least at lower temperatures – by addition of associative thickener (**Fig. 6D**).

What could be the consequences of changing the temperature of such micellar solutions? Higher temperature means everything is moving faster (Brownian motion, exchange kinetics). For a Maxwell fluid this means (in case there are no other temperature-induced structural changes to the micelles) that the entire frequency spectrum moves to the right – the crossover frequency ω_c is shifted to higher frequencies which means a decrease in relaxation time.

Another aspect when discussing temperature effects is changes in entropy: Entropy introduces a degree of randomness through bending of the cylindrical micelles – conformational entropy (similar to conformational entropy of polymer chains). Additionally, the entropy gain associated with more end-caps (i.e. increasing the number of micelles) is greater than that of branching points. More important than entropic effects, however, are changes in equilibrium phase diagram of the surfactants, caused by the temperature dependence of their spontaneous curvature (CPP). As long as we are above Krafft point, the temperature dependence of solubility of ionic surfactants is negligible. Ethoxylates, however, have an inverse solubility behavior: The higher the temperature, the less stretched is the polymer, and there is a temperature at which the random coil collapses (Cloud Point).

Most dramatic is this effect in case of associative thickeners, which are higher molecular weight ethoxylates carrying a hydrophobic anchor group at each end. The temperature dependent coil dimensions of associate thickeners play a major role for their thickening performance. In formulations consisting of associated spherical micelles (**Fig. 6D** and **7A**), the drop in viscosity at elevated temperatures can be dramatic.

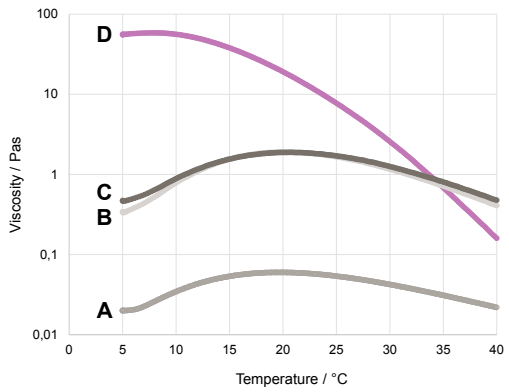


Fig. 6 Viscosity as a function of temperature for a model surfactant formulation: A) 9% SLES/3% CAPB/0.7% NaCl/pH 5.2; B) + 0.7% NaCl; C) + 0.9% hydrophobic thickener ANTIL® SPA 80 (Isostearamide MIPA (and) Glyceryl Laurate); D) + 0.5% associative thickener ANTIL® 141 (PEG-55 Propylene Glycol Oleate)

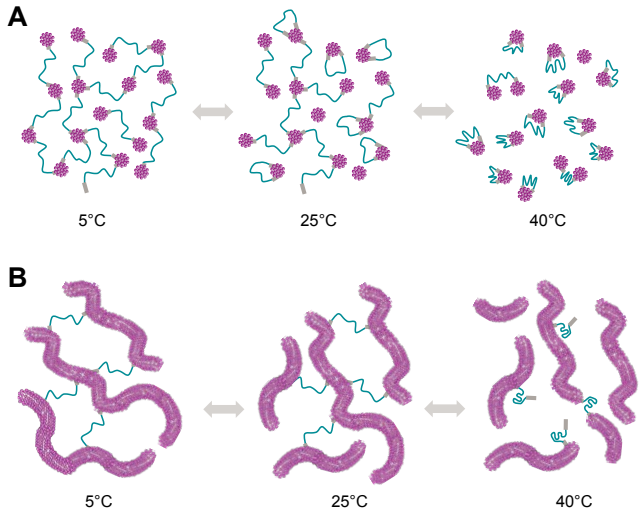


Fig. 7 Schematic representation of the temperature-induced changes occuring in thickened surfactant formulations: A) Associative thickener connecting spherical micelles B) Associative thickeners connecting worm-like micelles.

Therefore it is often advantageous to use a combination of hydrophobic and associative thickeners, since at elevated temperatures the drop in viscosity is not that pronounced, as there are still the (although rather short) worm-like micelles present (**Fig. 7B**).

Effect of chain length distribution

The same model system can be used to demonstrate the effect of alkyl chain length distribution on viscosity and rheology: 9% SLES/3% C_{xx}-APB/2% NaCl/pH 5.5 with three different alkylamidopropyl betaines (APB) of defined alkyl chain length: C_{10}-, C_{12}- and C_{14}-APB (**Fig. 8**). In case of C_{14}-APB, the shear thinning typical of worm-like micelles can be seen: In the shear field, the worm-like micelles are not present as random coils anymore, but orient in the direction of flow, and hence the viscosity drops. Replacing C_{14}-APB by C_{10}-APB, the viscosity (shear rate ramp, **Fig. 8A**) drops by a factor of 1000. The origin of this dramatic decrease can be understood by the rheological measurements shown in **Fig. 8B**: On the one hand, the structural relaxation time t_R is for C10-APB 100× shorter than for C_{14}-APB; this is a consequence of the faster exchange kinetics due to higher solubility/CMC. On the other hand, the plateau modulus (= crosslink density) is factor 10 lower. The combination of these two effects leads to the drop in viscosity by a factor of 1000. This example shows that the alkyl chain length distribution of the surfactants should always be considered when encountering "mysterious" viscosity effects.

After a discussion of the basic principles and those "easy" examples, the next step would be to use this know-how to build viscosity in more "green" formulations which are typically known to be hard-to-thicken.

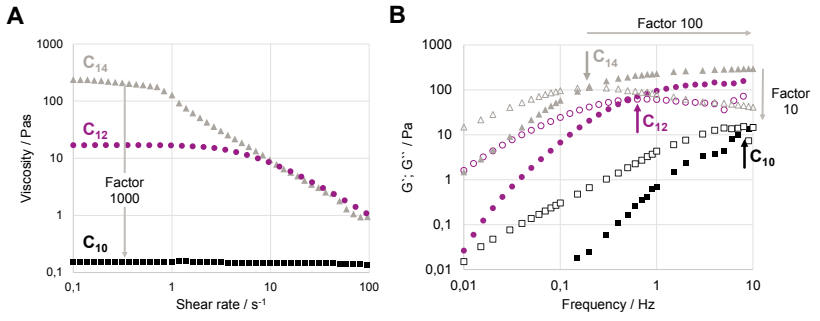


Fig. 8 Effect of surfactant alkyl chain length on rheological behavior of a formulation consisting of worm-like micelles: 9% SLES/3% CxxAPB/2% NaCl/pH 5.5; A) Viscosity as a function of shear rate; B) Storage (G'; closed symbols) and Loss (G"; open symbols) modulus as a function of frequency.

Conclusion

Thickening of surfactant formulations by utilizing worm-like micelles seems to be pretty easy at first sight, but it is rather complex since all changes in surfactant and co-surfactant levels, salt, pH will have an effect on length, stiffness and morphology of the micelles, and therefore on the macroscopic flow behavior. As a consequence, some might be tempted to consider thickening of surfactant formulations to be an art, but actually it is rather based on science; all phenomena no matter how counterintuitive they might seem at first sight can be logically explained using physical chemistry.

Remark

The inspiration to this paper came from discussions with our colleagues in Innovation Management Rinse-Off, specifically *Uta Kortemeier* and *Stefan Liebig*.

References

[1] *U. Kortemeier, J. Venzmer, A. Howe, B. Grüning,* and *S. Herrwerth*, "Thickening Agents for Surfactant Systems," SOFW-Journal, vol. 136, no. 3, pp. 30–38, 2010.

[2] *J. N. Israelachvili, D. J. Mitchell,* and *B. W. Ninham,* "Theory of Self-Assembly of Hydrocarbon Amphiphiles into Micelles and Bilayers," J. Chem Soc. Faraday Trans. II, vol. 72, pp. 1525–1568, 1976.

[3] *D. Danino, A. Bernheim-Groswasser,* and *Y. Talmon,* "Digital cryogenic transmission electron microscopy : an advanced tool for direct imaging of complex fluids," Colloids Surfaces A, vol. 185, pp. 113–122, 2001.

[4] *L. M. Walker,* "Rheology and structure of worm-like micelles," Curr. Opin. Colloid Interface Sci., vol. 6, no. 5–6, pp. 451–456, 2001.

[5] *J. Kleinen* and *J. Venzmer,* "Streaming potential measurements to understand the rheological properties of surfactant formulations containing anionic and zwitterionic surfactant," J. Cosmet. Sci., vol. 67, no. 2, pp. 59–70, 2016.

[6] *S. A. Rogers, M. A. Calabrese,* and *N. J. Wagner,* "Rheology of branched wormlike micelles," Curr. Opin. Colloid Interface Sci., vol. 19, no. 6, pp. 530–535, 2014.

[7] *P. G. de Gennes,* Scaling Concepts in Polymer Physics. Ithaka and London: Cornell University Press, 1979.

contact

Jochen Kleinen
Joachim Venzmer | joachim.venzmer@evonik.com

Evonik Operations GmbH
Research Interfacial Technology
Goldschmidtstr. 100 | 45127 Essen | Germany

mibelle GROUP biochemistry

SantEnergy™
Energizing hair follicles

SantEnergy™ is a distinctive polyphenol-rich extract that is sustainably obtained by wild harvesting from the aerial parts of the Yerba Santa plant. It energizes the hair from the root through a caffeine-like effect and optimally protects the follicles from oxidative damage. This increases both the hair growth as well as the hair density.

SantEnergy™ was shown to

- energize hair follicles through a caffeine-like effect
- protect hair follicles from oxidative stress
- delay aging of hair follicles
- increase both hair growth and hair density

SWISS QUALITY PRODUCT

www.mibellebiochemistry.com

Mibelle AG Biochemistry, 5033 Buchs/Switzerland, Phone +41 62 836 17 31

Protein Balance, a Key Player in Anti-Aging

M. Coirier, P. Rouaud-Tinguely, E. Aymard, E. Lasjaunias, B. Closs

abstract

Within cells, the Endoplasmic reticulum (ER) plays the key role of factory for proteins, essential molecules to ensure the body's vital mechanisms. In fact, it performs two fundamental functions by synthesizing proteins and ensuring their quality control. At the cutaneous level, functional proteins ensure firmness and radiance. However, a large number of situations of stress can interfere with this protein balance (or proteostasis). In response to this, the cell can trigger a "repair function", namely the UPR (Unfolded protein response).

Faced with these observations, SILAB Research decided to study the changes occurring in the ER during aging. This research work has shown for the first time that skin aging induces ER stress and limits the activity of the UPR pathway. This led to the development of ERISIUM®, an anti-aging active ingredient obtained from rice co-products that maintains proteostasis at the heart of the ER for an anti-wrinkle and radiance booster effect.

Introduction

In cosmetics, anti-aging strategies are at the core of products development, since this theme has always been and will continue to be one of the major concerns of consumers. Among these strategies, detoxification is an essential approach gaining more and more ground. Based on the discoveries made in basic research, it can be addressed through various aspects, such as exposomes and autophagy for example. Today, it appears that proteostasis (protein balance within cells) is a very promising new pathway to be explored. Indeed, in the course of aging or after exposure to a stress such as UV, cell metabolism progressively slows down. Among the molecules impacted, proteins are particularly affected. Yet, they are indispensable components of the body that account for more than 25% of the constituents of the skin. The production of functional proteins, taking place at the heart of cells, more precisely in the Endoplasmic reticulum (ER), ensures the vital mechanisms of the organism. At the cutaneous level, this optimal functioning translates into a firm and radiant skin. It therefore appears essential to preserve the metabolic activity of skin cells, especially ER functionality, in the course of aging. Faced with this observation, SILAB Research has developed ERISIUM®, an anti-aging active ingredient that maintains protein balance at the heart of cells (also called cellular proteostasis).

Key role of the endoplasmic reticulum in protein balance

The ER: a true protein factory

Proteins are biological molecules that carry out a variety of roles required for the correct functioning of the organism. In order for each protein to be functional, it must be in its own correct 3D conformation, in other words be specifically folded [1]. These proteins are produced at the heart of cells, in the ER. In order to fulfill its role as a protein factory, this latter has the particularity of carrying out two complementary activities. First, it has a "production" activity since it is the site of protein biosynthesis and maturation. It therefore plays an active and essential role in the synthesis of macromolecules required to produce the matrix mesh. Second, this intelligent organelle has a "quality control" activity for newly synthesized proteins because it ensures their correct folding and their 3D conformation [2]. In physiological conditions, if the ER is in "good health", it appears as an essential organelle for protein balance (also called cellular proteostasis) [3] (**Fig. 1**).

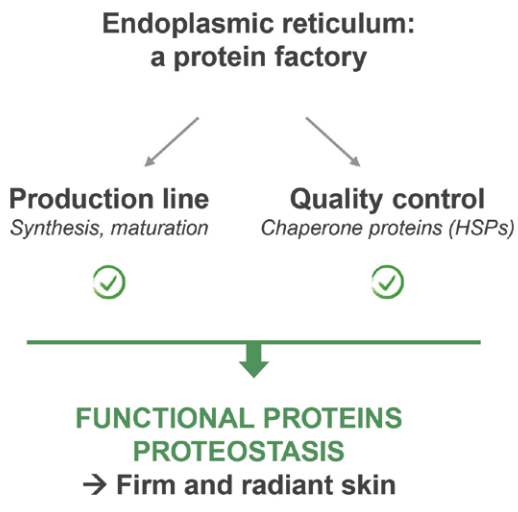


Fig. 1 Role and functions of the ER in physiological conditions.

The endogenous repair function of the ER: the UPR pathway

A large number of situations of stress can however interfere with protein maturation. In this case, newly synthesized proteins are no longer correctly folded, blocking their secretion out of the ER. When these proteins with incorrect conformation are produced in excess, they cannot be fully eliminated, ultimately accumulating in the lumen of the ER. The organelle is congested and its production and quality control activities are saturated: this is called ER stress [4]. In addition, the harmful accumulation of Reactive oxygen species (ROS) causes the oxidation of proteins [5]. Faced with this situation of stress, the cell can trigger a "repair function", called the Unfolded protein response (UPR), whose aim is to improve the functioning of the ER and its health by boosting its capacities [2, 3] (**Fig. 2**). At the molecular level, three signaling pathways involving three distinct transmembrane proteins are part of the UPR: IRE-1 (Inositol-requiring enzyme-1), ATF6 (Activated transcription factor 6) and PERK (Protein kinase RNA (PKR)-like endoplasmic reticulum kinase) [6, 7, 8].

Impact of aging on the ER stress and the UPR

Although these mechanisms are essential for cellular proteostasis, and thus to maintain a firm and radiant skin, no work had yet been done on ER stress or the UPR in the course of skin aging. The French company therefore got interested in this problematic, in particular in the dermis. To this end, ER stress and the expression of proteins triggering the UPR were studied in fibroblasts from young and old donors subjected to a UVA stress. This first-ever modeling study showed that aging is accompanied by an alteration of the UPR pathway (syntheses of IRE-1 and ATF6 reduced by 18% and 32% respectively) and a 43% increase of ER stress in response to a UVA stress. This study clearly demonstrated that following a UVA stress to old fibroblasts, ER stress is high and the UPR is deficient.

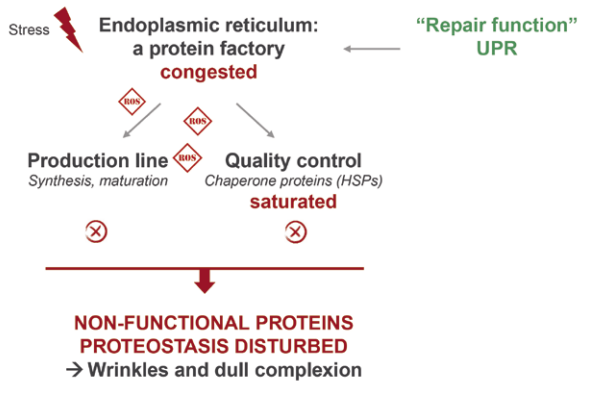


Fig. 2 ER stress and the UPR.

In order to determine the consequences of these disturbances on the metabolism of old fibroblasts, deficient activation of the UPR in young cells was mimicked using the siRNA technique. The results showed an induction of matrix degradation pathways, an activation of the inflammatory response and a reduction of key markers of matrix architecture after a UVA stress. The absence of activation of the UPR as seen in the course of aging therefore has a significant impact on the metabolism of fibroblasts. It fundamentally alters matrix dynamics *via* the mechanism of inflammaging (expression of pathways of inflammation and matrix degradation).

This novel work has shown for the first time the existence of a link between UPR-related proteostasis and matrix dynamics. Safeguarding the health of the ER is therefore a brand-new anti-aging strategy to preserve the functionality of the dermis and thereby maintain a firm and radiant skin. Based on its discoveries, SILAB has developed an anti-aging active ingredient from co-products of rice, targeting the endoplasmic reticulum, in order to reactivate the metabolism of old fibroblasts for an anti-wrinkle and radiance booster effect.

Target cellular proteostasis for an anti-aging performance

Co-products of rice: a source of bio-inspiration

To develop its novel concept of maintaining cellular proteostasis through the UPR, SILAB Research targeted natural raw materials rich in proteins. Indeed, just as skin cells, the ER of plant cells rich in proteins can be subjected to a stress [9] and in response to it, plant cells also activate their UPR pathways in order to ensure their production of functional proteins [10]. This ubiquitous and conserved mechanism is found in reproductive tissues such as rice germ, which can activate the UPR *via* homologues of IRE-1 and ATF6 [11]. The principal result is an induction of the expression of genes coding for ER chaperone proteins, required for the quality control of proteins.

In this context, the rice (*Oryza sativa L.*) (**Fig.3**) was selected, and especially its outer layers (bran + germ). These co-products, resulting from the bleaching of whole grain rice into white rice, are natural sources of essential nutrients (proteins, fibers, inorganic salts, etc.).

Scientific work on the UPR pathway in rice has shown the presence of signal peptides whose enriched sequence in leucine and valine is known to target stressed ER [11, 12]. In this context, the company has developed a unique enzymatic bioengineering process to specifically extract these peptides of interest.

Fig.3 Rice (*Oryza sativa L.*).

Restoring the UPR pathway for the production of functional proteins

Research work conducted *in vitro* enabled to determine the capacity of these peptides to reactivate the UPR pathway and thereby reducing the endoplasmic reticulum stress. Indeed, tested at 0.5% on normal human fibroblasts from old donors subjected to a UVA stress, the *Oryza sativa* active restores the UPR by boosting the synthesis of IRE-1 (+34%, $P < 0.05$) and of ATF6 (+32%, $P < 0.05$). Tested at 1%, the *Oryza sativa* active thereby leads to a reduction of ER stress by 33% ($P < 0.01$) (**Fig.4**).

In addition, ER stress and inflammation are intimately linked. Indeed, SILAB's modeling work has shown that in case of ER stress and deficient UPR, the expressions of interleukins (IL-) 1α, 1β and 8 are significantly increased. Tested at 0.5% on fibroblasts from old donors subjected to UVA irradiations, the *Oryza sativa* active significantly reduces the expression of

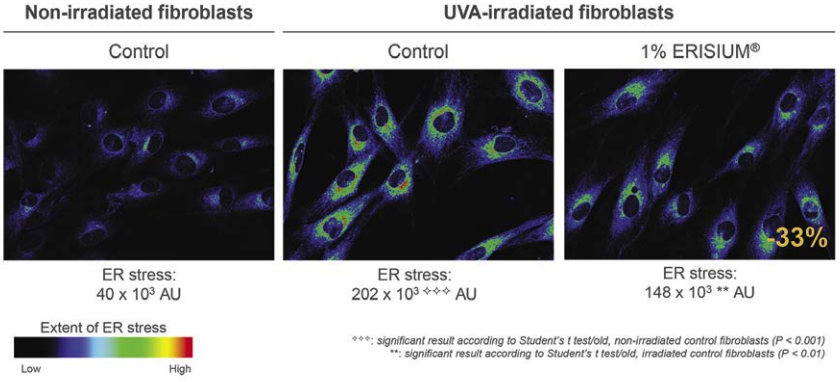


Fig.4 Visualization of the fluorescence emitted by the ER tracker probe, transcribing ER stress, in the presence or not of treatment with ERISIUM®.

genes coding for IL-1α, IL-1β and IL-8 by 58%, 49% and 72% respectively. It also decreases the secretion of IL-8 by 75% ($P < 0.01$) and pro-MMP1 by 48% ($P < 0.001$). The *Oryza sativa* active therefore limits the inflammaging process caused by UVA irradiations in aged cells.

All these elements result in restoring the matrix dynamics, with a significant increase of the production of perlecan (+42%, $P < 0.05$) and of the collagen I network (+37%, $P < 0.05$).

With its action at the heart of the endoplasmic reticulum, ERISIUM® maintains cellular proteostasis, thereby protecting the dermis from the harmful effects of aging.

Improving skin quality

In order to confirm the *in vitro* data on matrix dynamics, the company has developed a novel modeling study combining LC-OCT and artificial intelligence to predict the quality of the dermal matrix *in vivo*. It was conducted on three groups of volunteers (young, old and not photoexposed, old and photoexposed). This study showed that in the old, non-photoexposed group, there was a significant 18% decrease ($P < 0.001$) of dermal matrix quality compared to the young group. In addition, in the old, photoexposed group, there was a significant 17% decrease ($P < 0.01$) of matrix quality compared to the old, non-photoexposed group. These data therefore confirmed the impact of chronological aging and photoexposure on the quality of the dermal matrix. Then, a second modeling step was carried out in the old, photoexposed subjects to statistically determine the volume of dermis altered after UV exposure. This analysis showed the presence of a mean volume, 53 µm thick under the dermal-epidermal junction where the dermis is significantly more altered.

In this context, the ability of the *Oryza sativa* active formulated at 2.5% in an emulsion to improve skin quality was tested in the conditions previously defined (panel, dermis thickness, protocol). After 56 days of twice daily application, the *Oryza sativa* active improves the quality of the dermis by 20% in comparison to the placebo, a result observed in 100% of the volunteers.

The improvement of skin quality leads to an anti-wrinkle and radiance booster effect, which was confirmed on a Caucasian panel and an Asian panel, both old and photoexposed. Indeed, after 56 days of twice daily application, a visual scoring by trained evaluators using digital photos highlighted that the *Oryza sativa* active, formulated at 2.5% in an emulsion, significantly reduces the stage of wrinkles by 12% ($P < 0.01$) in Caucasian volunteers (on the crow's feet) (**Fig. 5**), and by 9% ($P < 0.01$) in Asian

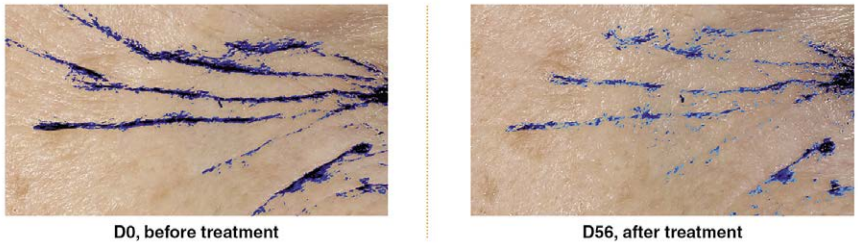


Fig. 5 Effect of ERISIUM® on the crow's feet wrinkles after 56 days of twice daily application on one half of the face (Caucasian panel).

volunteers (on the nasolabial fold) (**Fig. 6**). A subjective evaluation confirms these data: at the end of the treatment, 100% of the Caucasian volunteers feel their skin smoother and 100% of the Asian volunteers consider their complexion radiance revived and their wrinkles and fine lines smoothed.

Conclusion

Based on its 35 years of expertise in skin biology, SILAB has been able to identify an unprecedented concept, proteostasis in skin aging, and has developed two innovative *in vitro* and *in vivo* modeling studies. Moreover, the company, with its deep understanding of nature, has conducted a precise identification of the raw material fitting its concept: co-products of rice that also further supports its eco-responsible philosophy. Finally, SILAB perfectly masters non-denaturing manufacturing processes, enabling it to conduct a unique enzymatic bioengineering to extract and use beneficially plant peptides that can combat ER stress.

With its action at the heart of the endoplasmic reticulum, the resulting *Oryza sativa* active acts as a guardian of cellular proteostasis and therefore provides a comprehensive anti-aging response. It restores the UPR pathway weakened in the course of skin aging and preserves the health of the ER after a UVA stress. It reduces inflammaging and restores the matrix dynamics of mature skin. This re-equilibrating action improves the quality of the skin for an anti-wrinkle and radiance booster effect.

A patented solution of natural origin at 99% (ISO 16128), ERISIUM® (INCI name: *Oryza sativa* (Rice) Extract) is available in aqueous solution (recommended amount: 0.5 to 2.5%). It respects biodiversity regulations and is compliant with international cosmetic regulations (Europe, United States, Japan, China, etc.).

References

[1] *Tigges J, Krutmann J, Fritsche E, Haendeler J, Schaal H, Fischer JW, et al.* The hallmarks of fibroblast ageing. Mechanisms of Ageing and Development. 2014 Jun 1;138:26–44.

[2] *Ellgaard L, Helenius A.* Quality control in the endoplasmic reticulum. Nat Rev Mol Cell Biol. 2003 Mar;4(3):181–91.

[3] *Park K, Lee SE, Shin K-O, Uchida Y.* Insights into the role of endoplasmic reticulum stress in skin function and associated diseases. FEBS J. 2019;286(2):413–25.

[4] *Farrukh MR, Nissar UA, Afnan Q, Rafiq RA, Sharma L, Amin S, et al.* Oxidative stress mediated Ca(2+) release manifests endoplasmic reticulum stress leading to unfolded protein response in UV-B irradiated human skin cells. J Dermatol Sci. 2014 Jul;75(1):24–35.

[5] *Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, et al.* An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell. 2003 Mar;11(3):619–33.

[6] *Foufelle F, Ferré P.* La réponse UPR: Son rôle physiologique et physiopathologique. médecine/sciences. 2007 Mar;23(3):291–6.

[7] *Ron D, Walter P.* Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007 Jul;8(7):519–29.

[8] *Pallet N, Fougeray S, Beaune P, Legendre C, Thervet E, Anglicheau D.* Endoplasmic reticulum stress: an unrecognized actor in solid organ transplantation. Transplantation. 2009 Sep 15;88(5):605–13.

[9] *Vitale A, Boston RS.* Endoplasmic reticulum quality control and the unfolded protein response: insights from plants. Traffic. 2008 Sep;9(10):1581–8.

[10] *Wakasa Y, Yasuda H, Oono Y, Kawakatsu T, Hirose S, Takahashi H, et al.* Expression of ER quality control-related genes in response to changes in BiP1 levels in developing rice endosperm. Plant J. 2011 Mar;65(5):675–89.

[11] *Lu S-J, Yang Z-T, Sun L, Sun L, Song Z-T, Liu J-X.* Conservation of IRE1-regulated bZIP74 mRNA unconventional splicing in rice (*Oryza sativa L.*) involved in ER stress responses. Mol Plant. 2012 Mar;5(2):504–14.

[12] *Cheon SA, Jung K-W, Chen Y-L, Heitman J, Bahn Y-S, Kang HA.* Unique Evolution of the UPR Pathway with a Novel bZIP Transcription Factor, Hxl1, for Controlling Pathogenicity of Cryptococcus neoformans. PLoS Pathog.

Fig. 6 Effect of ERISIUM® on the nasolabial fold wrinkles after 56 days of twice daily application on the entire face (Asian panel).

authors

Mélanie Coirier
Pauline Rouaud-Tinguely
Elodie Aymard
Emilie Lasjaunias
Brigitte Closs

SILAB
19240 Saint-Viance | France

hpci
CENTRAL &
EASTERN EUROPE

www.hpci-events.com

EXHIBITION & CONFERENCE

FOR THE COSMETIC AND DETERGENT INDUSTRY

22 - 23 SEPTEMBER 2021

WARSAW | POLAND

hybrid edition NEW!

- → brings together suppliers from every point of the production chain
- → gives brands and manufacturers of cosmetics and personal and home care products exclusive access to all services required for product development
- → shows new product ideas

Home and Personal Care Ingredients Exhibition and Conference

Central & Eastern Europe

Vincentz Network GmbH & Co. KG | P.O. Box 6247 | 30062 Hannover

VINCENTZ

A Japanese Zen Aesthetic-inspired Formulation with a Root-powered Active Ingredient

Energizing the Skin Cells to Face Daily Stresses and Recover Serenity

A. Werle, C. Stricane, S. Delaunois, B. Mignard, A. Lapeyre, A. Guillaumin, N. Rombaut, J. Brouillot, M. Frechet, H. Chajra

abstract

As we are living in a world full of uncertainties, where we are challenged by the coming together of environmental, health and employment crises, mental and physical stresses tend to overwhelm us. These stresses have deleterious effects on human body and especially on skin. They lead to the impairment of cell bioenergetic functions, thus contributing to premature skin aging. To recover serenity, being inspired by Japanese traditions and their Zen aesthetic principles can be a new way to address daily stresses. This paper presents the concept of Zen aesthetics that allowed us to develop several formulations under the beauty concept called "Zenspiration". Among these formulations, the Energizing Water Cream is dedicated to re-energizing stressed cells. This paper also describes the skin biological benefits provided by an active ingredient called Rootness™ Energize, included in the Energizing Water Cream, and developed thanks to the plant milking technology. This extract from the roots of *Luffa cylindrica* demonstrated *in vitro* its ability to protect and stimulate cell bioenergetic functions while stimulating antioxidant defense systems and accelerating the renewal of key extracellular matrix components. A clinical study enrolling stressed women demonstrated that Rootness™ Energize improved skin elasticity and firmness in stressed women, leading to a reduction in wrinkles.

Introduction

Over recent years, we have become more and more conscious that we are living in a stressful world. Each individual is overwhelmed by a strong mental charge materialized by the fear of an uncertain future brought by the recent accumulation of simultaneous crises. Here are a few examples: sanitary crisis (covid-19…), political and economic crisis (retrenchment plan and company closings), and environmental crisis (climatic change, pollution…). Mental and physical stresses are known to be major triggers of adaptive reactions from our body, activating biological machinery and causing over-production of free radicals by the cells. This leads to the apparition of visible signs of fatigue and premature aging. To fight and mitigate the stress before it overwhelms us, consumers are seeking relaxing solutions and self-care has become predominant. Experiencing different beauty traditions and customs has become a popular way of relieving the daily worries, even for an hour. Taking Japan as an example, it is commonly known that over centuries the country has developed a strong culture around Zen Buddhism and meditation, a practice that makes people realize the emptiness of their existence and open the path to a liberated way of living. In Japan, Zen is so entwined in everyday life that it can be found in every layer of Japanese ethics and design. Complex and allowing multiple layers of meanings, Zen aesthetics are a constant source of inspiration. One principle is of peculiar importance: Wabi-Sabi. Roughly translated into "wisdom in natural simplicity", this concept calls for minimalistic aesthetics and a search for singularity, fitting with a quest for less stressful environments.

With the Japanese Zen aesthetics in mind, Clariant Active Ingredients created Sc[ai]turalist Zenspiration, a beauty concept built around sensorial excellence. It is composed of three formulations, each illustrating a different principle of Japanese Zen while inviting the senses to rise above the ordinary and to reinforce natural beauty:

- Kanso Zen principle was illustrated by the Simply Rebalancing Scalp Toner formulation. The word relates to the concepts of basic simplicity and the elimination of the ornate. Things expressing simplicity are by nature truthful and reserved. Therefore, the "Kanso" Simply Rebalancing Scalp Toner is a minimalistic formula containing only 16 necessary ingredients to create a light sensation on application.
- Shibui, used to describe the beautiful simplicity perceivable through time and experience, was illustrated by the On-the-Glow Solid Serum formulation. This solid serum was formulated in a practical stick format to reduce the amount of water used and time spent on beauty routines.
- Datsuzoku is the third Zen principle illustrated in the Zenspiration concept by the Energizing Water Cream. The word describes the feeling of serenity and happiness when one realizes the daily stresses of life are irrelevant to one's true self. This formulation brings hydration and energy to the skin for a fresh and restored complexion.

This last formulation features Rootness™ Energize, an active ingredient extracted from the roots of *Luffa cylindrica*, known as "hechima" in Japan, a plant from the Cucurbitaceae family. Rootness Energize is produced thanks to a specific process

Fig. 1 Close-up view of Luffa cylindrica roots grown aeroponically

called Plant Milking that allows rare active molecules to be extracted from plant roots without destroying the plant [1]. The plant milking process complies fully with the rules of Zen as it respects the life cycle of the plant.

Results and discussion

Rootness™ Energize has a unique composition enriched in bryonolic acid

We developed a plant extract from *Luffa cylindrica* roots enriched in bryonolic acid (BA) because this molecule is a plant secondary metabolite known both to exhibit interesting biological properties such as anti-inflammatory and antioxidant activities [2], and also support important cellular processes for the plant, such as resistance to pathogens or environmental stress [3; 4]. Thus, capturing the plant's ability to fight stress thanks to BA was the starting point for the development of this new active ingredient. BA is present exclusively in the roots of certain Cucurbitaceae species. In these plants, the accumulation of bryonolic acid in the roots is promoted by mychorrizal fungi colonization.

To determine the variety of Cucurbitaceae to use in order to produce an extract containing bryonolic acid, several varieties were cultivated in an aeroponic culture system (**Fig. 1**). The concentration of BA in dried roots and after ethanol extraction was then measured (**Tab. 1**). It appeared that in comparison to other Cucurbitaceae species, *Luffa cylindrica* was the variety that produced the highest quantity of bryonolic acid, hence its selection.

In order to confirm the benefits of aeroponic cultivation on bryonolic acid concentration versus traditional soil culture, seedlings of *Luffa cylindrica* were cultivated in pots with soil in a greenhouse. The content of bryonolic acid was analyzed. BA was detected in these roots; however, its amount was under the HPLC limit of quantification. This confirms the influence of aeroponic culture for improving the bryonolic acid content in roots of *Luffa cylindrica*.

Unfortunately, even though the native root extract of *Luffa cylindrica* contains the highest rate of bryonolic acid, its concentration remains low. Thanks to the Plant Milking technology, the roots were stimulated with a nutrient solution which allowed us to obtain a strong root growth and to the increase their content of bryonolic acid. After two cycles of production and harvest, the yield of bryonolic acid (mg/g of dried roots) in the stimulated roots was multiplied by 7 and the production of root (g/m²) was multiplied by 1.5 for the second harvest compared to non-stimulated roots (**Tab. 2**). Therefore, the total bryonolic acid yield (mg bryonolic acid/m²) was multiplied by 11.

From these stimulated roots of *Luffa cylindrica*, we obtained an extract rich in bryonolic acid. Named Rootness™ Energize, it demonstrated strong capabilities to stimulate skin cells' bioenergetic pathways under basal and stress conditions.

The contents of bryonolic acid (BA) are indicated in Tab. 1 species	Provider country	BA concentration (mg/mL of ethanol)	BA concentration (mg/g of dried roots)
Ipomea nil	United Kingdom	0.0	0.0
Phaseolus lunatus	USA	0.0	0.0
Luffa operculata	United Kingdom	14.7	1.5
Cucumis metuliferus	France	15.7	1.6
Cichorium intybus	France	0.0	0.0
Benincasa hispida	France	13.5	1.4
Coccinea grandis	France	0.0	0.0
Momordica charaptia	United Kingdom	0.0	0.0
Luffa cylindrica roots	France	48.0	4.8
Luffa cylindrica leaves	France	0.0	0.0

Tab. 1 Content of bryonolic acid in different plant species.

Condition		Without stimulation	With stimulation
First cut	Bryonolic acid (mg/g of dried roots)	0.70	1.84
	Weight of dried roots (g/m²)	39	52
Second cut	Bryonolic acid (mg/g of dried roots)	1.03	7.03
	Weight of dried roots (g/m²)	27	45

Tab. 2 Effects of the stimulation on the weight of dried roots obtained and the amount of bryonolic acid after the first and the second cultivation cycles.

Rootness™ Energize preserves and boosts stress impaired mitochondrial bioenergetic cellular functions

Cells exposed to stress require high amounts of energy to maintain homeostasis and ensure proper functioning of the biological mechanisms required to adapt to this stress [5]. This energy, provided thanks to the ATP molecule, is mainly produced through the respiratory chain in the mitochondria and relies on a well-functioning mitochondrial respiration process. In order to study the properties of Rootness Energize to boost energetic functions of the cells in basal and stress conditions, we used the Seahorse technology. This equipment allows us to determine three parameters which are the most relevant when assessing mitochondrial respiration: maximal respiration, spare capacity and ATP production. Maximal respiration is the higher respiration rate, indicating efficient bioenergetic profile and spare capacity is the mitochondrial reserve respiratory capacity. These parameters reflect the ability of cells to increase their respiration to react to stress or respond to an energy need. ATP production reveals the ability of mitochondria to produce energy.

Normal human dermal fibroblasts (NDHF) were used as a study model as these cells play a crucial role in dermis homeostasis. Besides, the dermal compartment is strongly impacted by premature aging induced by external stressors such as UV or pollutants [6; 7]. NDHF were chemically stressed and pretreated or not with Rootness Energize at 1%. As expected, the chemical stress impairs mitochondrial respiration as shown by the dramatic decrease of maximal respiration parameter (-93%), spare capacity parameter (-96%) and ATP production (-100%) (**Fig. 2**). In contrast, when cells are exposed to the same stress but pre-treated with Rootness Energize, the mitochondrial respiration is preserved and even boosted, thus revealing the strong protective effect of the active ingredient.

Rootness™ Energize stimulates intrinsic antioxidant defense cellular mechanism impaired by stress

Stresses such as UVB, pollutants (cigarette smoke, particulate matters…) or emotional stress, are involved in ROS overproduction as well as impairment of the cellular antioxidant mechanism by significatively reducing the levels of GSH (Glutathione and GSH dependent enzymes), superoxide dismutase and catalase [8]. Using a 3D reconstructed human epidermis model, we found that Rootness Energize topically applied at 0.5% stimulates the antioxidant intrinsic defense cellular mechanism at the transcription level (**Fig. 3**). Indeed, mRNA transcripts coding for two enzymes belonging to the intrinsic anti-oxidative system, glutathione peroxidase 2 and peroxiredoxin-2 were significantly upregulated: glutathione peroxidase 2 expression was increased by 1.33-fold ($p<0.05$) and peroxiredoxin-2 expression was increased by 1.20-fold ($p<0.05$). The glutathione peroxidase enzymes are responsible for the protection of the cell from oxidative damages [9]. Peroxiredoxin 2 is an enzyme which catalyzes the reduction of hydrogen peroxide and organic hydroperoxides, thus protecting the cells against oxidative stress by detoxifying peroxides [10]. The proteomic analysis performed on a human skin explant model pretreated topically by Rootness Energize at 1% confirmed these transcriptional results. Indeed, in this skin model, we showed a significant up-regulation of the glutathione antioxidant system (**Tab. 3**). Interestingly, we found that the

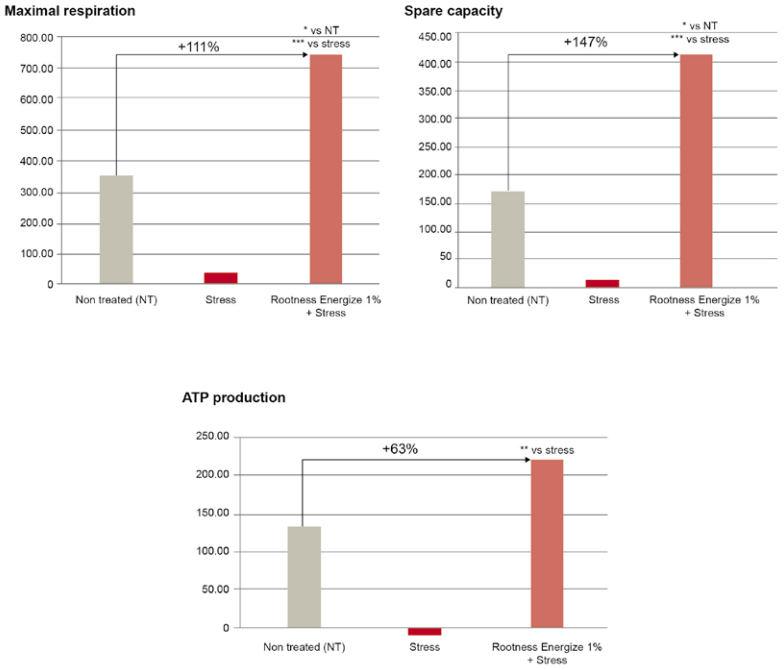
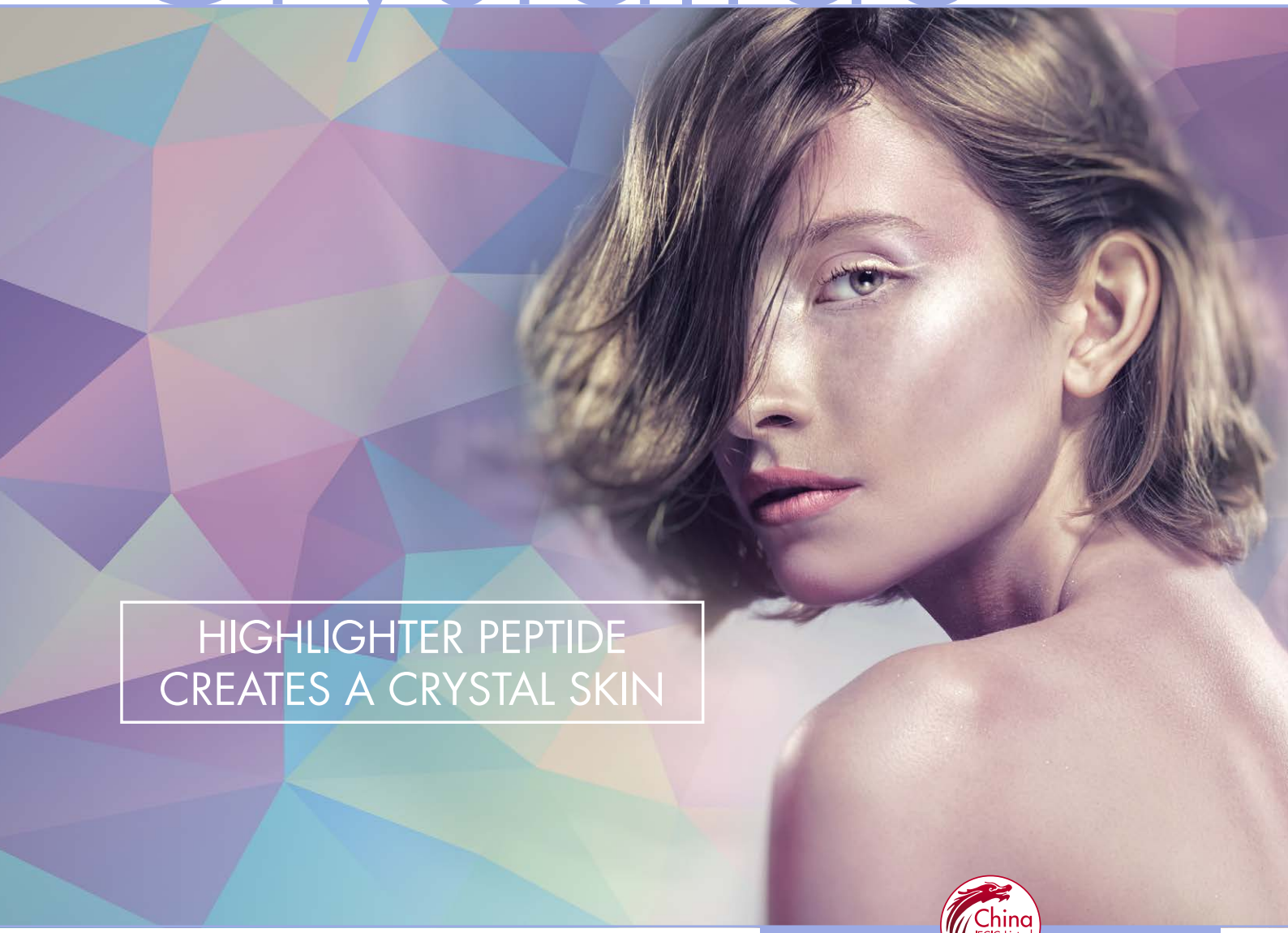


Fig. 2 Maximal respiration, spare respiration capacity and ATP production levels of mitochondria in NDHF from a 44 years old woman in basal conditions (grey), exposed to a chemical stress (black) or exposed to a chemical stress with an 18-hours treatment of Rootness Energize (pink). Results are expressed in Oxygen Consumption Rate pmol/min/µg prot. Statistical analysis was performed using a Welch's ANOVA. ***: $p<0.001$; **: $p<0.01$; *: $p<0.05$; #: $p<0.1$.

Proteins	Fold-change	p-value
Glutathione reductase, mitochondrial	1.53	*
Glutathione S-transferase omega 1	1.50	**
Glutathione S-transferase LANCL1	1.33	*
Deaminated glutathione amidase	1.62	*
Superoxide dismutase [Cu-Zn]	1.73	*

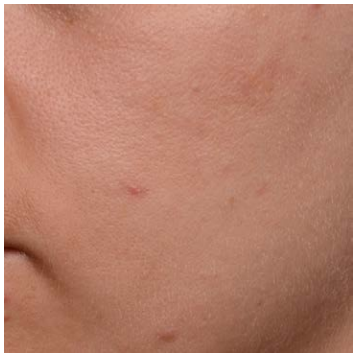
Tab. 3 Protein expression analyzed by mass spectrometry on skin explant model treated 7 days with a formula containing 1% of Rootness Energize. **: $p<0.01$ *: $p<0.05$.

Crystalide®

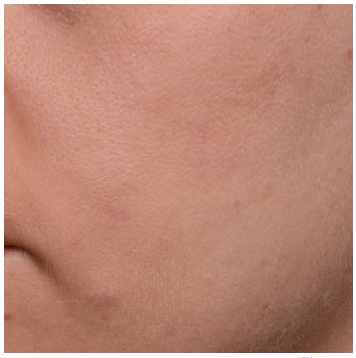


HIGHLIGHTER PEPTIDE CREATES A CRYSTAL SKIN

China IECIC Listed



BEFORE



AFTER **6 WEEKS** APPLYING **CRYSTALIDE™** 3 %

volunteer #18

- New studies on Asian panel confirm the benefits on skin radiance and luminosity.
- Crystalide® upgrades the protective activity of α-cristallin *in vitro* to ensure cell proteostasis.
- Crystalide® bio-harmonises the epidermis renewal leading to a soft-polish effect and a dewy finish.
- The skin looks clear, smooth and glowy just like a piece of crystal.

EUROPE - Sederma SAS Tel +33 1 34 84 10 10 email sederma@sederma.fr www.sederma.com
Sederma GmbH Tel +49 21 57 817318 email sederma@sederma.de www.sederma.com
NORTH AMERICA - Sederma Inc. Tel +1 609 212 2500 email sederma-usa@croda.com www.sederma.com
ASIA PACIFIC email pc-asia@croda.com www.sederma.com

Smart science to improve lives™

Follow us on

sederma

Scan the code for free* sample Part of Croda international Plc.

*Samples will be sent at Sederma's discretion

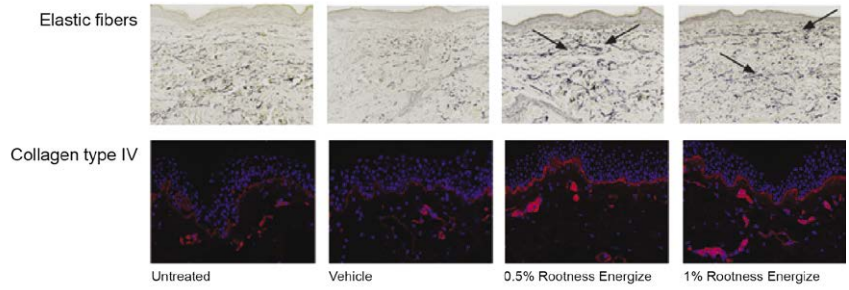


Fig. 3 Dark and pink staining on skin explants exposed to different doses of Rootness Energize, showing resp. the elastic fibers and collagen type IV.

superoxide dismutase [Cu-Zn], a metalloprotein that catalyzes the dismutation of oxygen radical into molecular oxygen and hydrogen peroxide [11], was also significantly up-regulated. These results demonstrate the active is able to reinforce the protection of cells against the consequences of stress.

Rootness™ Energize stimulates the renewal of key extracellular matrix components to support skin homeostasis

The skin extracellular matrix is a complex network of collagenous and non-collagenous components. It is responsible for the overall skin quality. Specifically, elastic fibers are responsible for skin elasticity, while collagen fibers are responsible for skin firmness. Collagen IV, a type of collagen found in the basement membrane zone, has a structure that allows it to be more pliable and kinked than other collagen types which allows it to form sheets, the primary structural form found in the dermis-epidermis junction [12]. Regular renewal of these components is necessary to maintain proper skin homeostasis.

Nevertheless stress, either psychological or physical (pollution, UV, cigarette smoke…), is known to impact the extracellular matrix by degrading components of the dermis-epidermis junction. UV induces the production of matrix metalloproteinases which degrade dermal collagen fibers and elastic fibers in the dermis [13]. Cigarette smoke was demonstrated to cause abnormalities in elastic fibers that become more numerous, shorter, wider and fragmented. It also inhibits collagen production [14].

The effect of Rootness energize on these two major skin components (elastic fibers and collagens specifically collagen type IV) was investigated by the use of a human skin explant model from a 42-year-old woman. The skin explants were treated daily for 7 days with a cosmetic cream containing either 0.5% or 1% Rootness energize (topical application). At the end of the culture, skin samples were analyzed by histology in order to allow the visualization of elastic fibers thanks to modified Verhoeff's staining or by immunofluorescence technique in order to observe collagen type IV expression level. Elastic fibers appear dark by this staining method and collagen type IV appear in pink. staining. Interestingly, an increase in the dark and pink staining was observed in a dose dependent manner as the concentration of Rootness Energize increased in comparison with the placebo. This demonstrates the ability of Rootness Energize to promote the production of elastic fibers and collagen type IV (**Fig. 3**).

Rootness™ Energize increases skin elasticity and firmness in women exposed to stress

Chronic stress, either psychological or due to recurrent pollution or UV exposure, is known to be a trigger of premature aging with the accelerated apparition of wrinkles [13; 7]. To evaluate the capacity of Rootness Energize to mitigate the skin stress effect, we enrolled 20 working women aged 45 – 55, with an average of 51.8 years old, who described themselves as stressed, and who presented a lack of skin firmness and elasticity. Twice daily for 62 days they used a formula containing 1% Rootness Energize on one half-face and a placebo formula on the other half-face. The skin mechanical properties were measured with a cutometer at day 28 and day 62. Moreover, wrinkles' improvement was followed by pictures.

As shown in **Fig. 4**, Rootness Energize significantly improves skin mechanical properties. Skin firmness, skin tonicity and skin elasticity were all significantly improved by Rootness Energize after 28 and 62 days of use. The effect observed was dependent on the duration of product used. Indeed, the longer the product is used the better the result. Skin firmness was improved by 12% after 28 days, and by 20% after 62 days. Skin tonicity was strengthened by 9% at day 28 and by 21% at day 62. Skin elasticity was also improved by 16% at day 28 and by 27% at day 62 thanks to Rootness Energize. For these three parameters, the placebo formulation had no significant effect.

Pictures of the volunteers taken at day 0 and day 62 show a visible improvement of the overall face condition, especially

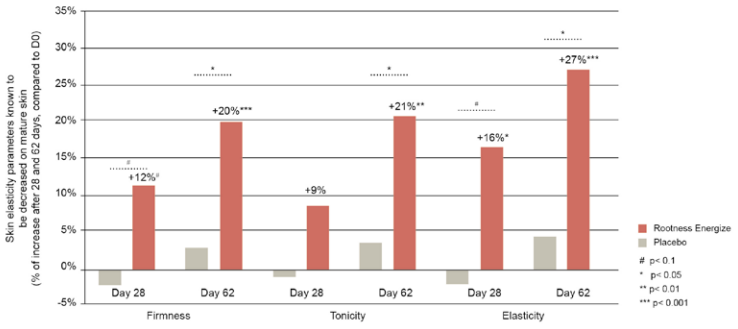


Fig. 4 Clinical measurement with cutometer of skin firmness, tonicity and elasticity after 28 and 62 of twice daily application a cosmetic cream containing either placebo or Rootness Energize (1%) on the face. Statistics: ***: p<0.001; **: p<0.01; *: p<0.05; #: p<0.1.

on wrinkles. **Fig. 5** represents the pictures of a woman who presented a tired face with dark circles and marked wrinkles on the crow's feet area, nasolabial folds and marionette lines at the beginning of the study. After 62 days, the effects of Rootness Energize were notably visible while placebo had no effect.

Conclusion

This work allowed us to demonstrate that it is clearly possible to improve and mitigate the visible and deleterious effects of stress on the skin using a cosmetic active ingredient. Indeed, we have demonstrated that Rootness Energize, a cosmetic active ingredient enriched in bryonolic acid thanks to a unique patented technology, the Plant Milking technology, is beneficial for the skin.

The anti-stress mechanism of action of Rootness Energize is through the stimulation of ATP production and up-regulation of intrinsic antioxidant systems known to be dampened under psychological and environmental stresses. Moreover, this cosmetic active ingredient promotes synthesis and maturation of elastic fibers and collagen type-IV, leading to visible improvements of the skin quality of active women exposed to stress. Finally, we demonstrated at the clinical level that these molecular effects are followed by clear visible effect on stressed skin. Skin is firmer, more tonic and more elastic after 2 months of Rootness Energize use.

This active ingredient is particularly suitable for formulations dedicated to dealing with the effects of psychological and environmental stresses on the skin, as exemplified by the Datsuzoku Energizing Water Cream inspired by the principles of the Zen aesthetics.

This experimental work also highlights that the Plant Milking technology allows possibilities to find new and strong activities in already well-known plants, to bring new solutions for customers who are looking towards tradition and reassurance in an uncertain and stressful environment.

Materials and methods

Plant extract studied

The seeds of *Luffa cylindrica* were purchased from a French supplier (Graines Baumaux, Mazirot, France). The seedlings are installed in aeroponic culture system and the plants cultured with a nutrient medium composed of 15/10/30

Fig. 5 VISIA pictures of volunteer 22 at day 0 and day 62 for each half-face: placebo and Rootness Energize 1%. Arrows point to the most visible effects on crow's feet, nasolabial folds and marionette lines.

N/P/K. Then, during 2 to 6 weeks, the plants are stimulated with a solution comprising less than 6% nitrogen. Roots were put to dry and extraction was done by maceration in a solution of dicaprylyl ether to obtain the extract named Rootness™ Energize.

Determination of cellular bioenergetic functions

We used the Seahorse XF Extracellular Flux Analyzer XF24 (Agilent Technologies) and the Seahorse XF Cell Mito Stress Test Kit (Agilent Technologies, ref 103015-100) according to the manufacturer's instructions. Six hours after NHDF seeding (44 years old woman), a chemical stress was added to the cells using 9% of 2-Methyltetrahydrofuran (MethylTHF, Carlo Erba) in presence or not of LCRE at 1% in culture medium (DMEM, Biowest). The stress and treatment lasted 18h, then Seahorse assay was performed. Each tested condition consisted of a minimum of 3 wells per experimental run. Seahorse assay consists in oxygen consumption rate measurement while successive administration of 1.0 µM oligomycin, 2.0 µM FCCP, and a combination of 1.0 µM rotenone and 1.0 µM antimycin A were mechanically done in the Seahorse XF Flux Analyzer. These toxins have the respective effects of inhibiting ATP-synthase (oligomycin), uncoupling oxidative phosphorylation (FCCP), inhibiting complex II and complex III (Rotenone and antimycine A). This modulates the oxygen consumption rate which allow itself the calculation of cellular bioenergetic parameters as important as maximal respiration, spare capacity or ATP production. Maximal respiration is the higher respiration rate, indicating efficient bioenergetic profile and spare capacity is the mitochondrial reserve respiratory capacity. These parameters reflect the ability of cells to increase its respiration to react to stress or respond to an energy need. ATP production reveals the ability of mitochondria to produce energy.

Transcriptomic analysis (RHE model)

The study was carried out on human reconstructed epidermises (RHE, StratiCELL, batch: AW0519/2). At the end of the RHE differentiation (day 14), formulated LCRE at 0.5% was applied topically (2 mg/cm²) for 24h as well as the placebo formulation. Formulations was prepared with Water, Ammonium Acryloyldimethyltaurate/VP Copolymer, Dicaprylyl Ether, Tocopherol, *Luffa cylindrica* Root Extract, Citric Acid, Sodium Citrate, Phenoxyethanol, Methylparaben, Ethylparaben, Fragrance. All the treatments were performed in triplicates (n=3). Non treated RHE were also cultivated in parallel. At the end of the topical treatment, RNAs were extracted using RNeasy Mini kit from Qiagen (Qiagen, 74106). Their concentrations and integrities were analyzed by spectrophotometry and capillary electrophoresis.
Gene modulation was analyzed thanks to Affymetrix human Clariom S arrays (Affymetrix, 902916 and 902917), according to manufacturer's instructions. The processing of the raw data was realized with software R (v3.2.3) and Bioconductor project (v3.2). The latest version of libraries provided by Affymetrix, constructed on version 19 of the human genome (UCSC Human genome 19), and the RMA method 4,5 were used to guide and perform the pre-treatments, and the sequences annotations.

Proteomic analysis of Skin explants

Skin explants were obtained from abdominal surgery (age 42 years old, woman, Caucasian, phototype III). Explants were stabilized in a proprietary medium at 37°C, 5% of CO_2 for 24h. Then, the formulated LCRE at 0.5% or 1% was applied topically (2 mg/cm²) as well as the placebo formulation. All the treatments were performed in triplicates. Non treated explants were also cultivated in parallel.
The application was renewed every day for 7 days. Each condition was done on 6 explants. Explants were snap-frozen on liquid nitrogen and were stored at -80°C until analysis for protein extraction. Once proteins from whole explants were extracted. Their concentrations were determined using the Bradford method and quality control of the extraction was validated by high resolution SDS-PAGE.
Mass spectrometry (MS) analyses were performed on a Dionex U3000 RSLC nano-LC system coupled to an Orbitrap Fusion mass spectrometer (Thermo Fisher Scientific). The MS data were analyzed using MaxQuant software using the Uniprot-Swissprot reviewed database (Uniprot-Swissprot, release 2019-05). Functional analyses of data from MaxQuant were done with Perseus software.

Skin staining and immunolabelling

Samples from the same experiments as for proteomic analysis were cut in two: one half was snap-frozen for cryosectioning and the other was formalin fixed for paraffin embedding.
For histological staining of elastic fibers, we performed a modified Verhoeff's staining using Bio-Optica 04-056802 kit on paraffin thin-sections. The staining was done according the manufacturer's instruction except for Van Gieson counterstaining which was omitted to improve elastin-to-background contrast. Collagen IV immunofluorescence analysis was done on cryosections. After blocking with 3% BSA at RT for 30 minutes, collagen IV antibody (Abcam, ref ab6586) was applied at 1/500 for 18 hours at 4°C. Secondary antibody (Invitrogen, Goat anti-Rabbit IgG Alexa fluor® 568, ref A11011) was then incubated at 1/500 for 2 hours at RT. Nuclei counterstain was done using Hoechst 33342 (Invitrogen, ref H3570) at 1/5000 for 10 minutes at RT.

Clinical investigation

A randomized double blind, placebo controlled clinical trial was conducted in France by Cosderma. The study was conducted in accordance with the principle of the Declaration of Helsinki and the guidelines of the International Conference

on harmonization Good Clinical Practice as applicable to a non-drug study. The volunteers gave written and informed consent. Twenty Caucasian females who gave their informed consent were enrolled in this clinical study. The study duration was 62 days. The volunteers were in the age range from 45 to 55 years, were active, stressed and presented a lack of skin firmness. Subjects applied the placebo and the product containing the active ingredient Rootness™ Energize at 1% twice a day on their half face. The **formulas** used were the following.

Pictures were taken with VISIA-CR before the first application of product and after 62 days of usage. The pictures allow visualizing the cutaneous features of the skin's whole face.

Mechanical skin parameters were assessed using a cutometer, before the use of the product, and after 28 or 62 days of use. The zone of skin measured was the temple.

The cutometer allows the measurement of the following parameters were measured:

- Firmness: total elongation (Uf = R0)
- Tonicity: immediate retraction (Ur)
- Elasticity: immediate elongation (Ue)

Statistical analyses were performed as following:

- Homogeneity of the areas at D0 (D0product vs D0placebo) by a Student test for paired series (threshold of risk of 5%) if the result of the normality test of Shapiro-Wilk was superior to 1%, or by a Wilcoxon test (threshold of risk of 5%) if the result of the normality test of Shapiro-Wilk was inferior to 1%,
- Evolution through time of each area (DiProduct vs D0product) by a univariate analysis of variance with one factor (factor time fixed, subjects in random) followed by a Dunnett test (threshold of risk of 5%) comparing the different experimental times with D0. These analyses were performed on the ranks if the result of the normality test of Shapiro-Wilk is inferior to 1%,
- Comparison of the (Di-D0) difference between the two areas [(Di-D0) product vs (Di-D0) placebo]: by a univariate analysis of variance with one factor (factor area fixed, subjects in random) (threshold of risk of 5%). This analysis was performed on the ranks according to the result of the normality test of Shapiro-Wilk at the threshold of 1%.

Conflict of interest

The authors state no conflict of interest.

References

[1] *Salwinski A, Warnault P, Guillaumin A, Duriot L, Droux J, Lapeyre A, Frechet M, Chajra H.* Roots – the Underexplored Source of Innovative Molecules Highlighted Thanks to PAT Plant Milking™ Technology. SOFW Mag. 2020 Sep and 146:2-10.

[2] *Gatbonton-Schwager TN, Letterio JJ, Tochtrop GP.* Bryonolic acid transcriptional control of anti-inflammatory and antioxidant genes in macrophages *in vitro* and *in vivo*. J Nat Prod. 2012 and 75(4):591-598.

[3] *Tabata M, Tanaka S, Cho HJ, et al.* Production of an anti-allergic triterpene bryonolic acid, by plant cell cultures. Journal of Natural Products. 1993 Feb and 56(2):165-174.

[4] *Akiyama K, Hayashi H.* Arbuscular mycorrhizal fungus-promoted accumulation of two new triterpenoids in cucumber roots. Biosci Biotechnol Biochem. 2002 Apr and 66(4):762-9.

[5] *Swan CL, Sistonen L.* Cellular stress response cross talk maintains protein and energy homeostasis. EMBO J. 2015 and 34(3):267-269.

[6] *Debacq-Chainiaux F, Leduc C, Verbeke A, Toussaint O.* UV, stress and aging. Dermatoendocrinol. 2012;4(3):236-240. doi:10.4161/derm.23652.

[7] *Parrado C, Mercado-Saenz S, Perez-Davo A, Gilaberte Y, Gonzalez S, Juarranz A.* Environmental Stressors on Skin Aging. Mechanistic Insights. Front Pharmacol. 2019 and 9, 10:759. Published 2019 Jul.

[8] *Chen Y, Lyga J.* Brain-skin connection: stress, inflammation and skin aging. Inflamm Allergy Drug Targets. 2014 and 13(3):177-190.

[9] *Flohé, L.* Glutathione peroxidases. Selenoproteins and Mimics p. 1–25, 10.1007/978-3-642-22236-8_1 (2012).

[10] *Perkins A, Nelson KJ, Parsonage D, Poole LB, Karplus PA.* Peroxiredoxins: guardians against oxidative stress and modulators of peroxide signaling. Trends Biochem Sci. 2015 and 40(8):435-445.

[11] *Mondola P, Damiano S, Sasso A, Santillo M.* The Cu, Zn Superoxide Dismutase: Not Only a Dismutase Enzyme. Front Physiol. 2016 and 29., 7:594. Published 2016 Nov.

[12] *Abreu-Velez AM, Howard MS.* Collagen IV in Normal Skin and in Pathological Processes. N Am J Med Sci. 2012 and doi:10.4103/1947-2714.92892, 4(1):1-8.

[13] *Dunn JH, Koo J.* Psychological Stress and skin aging: a review of possible mechanisms and potential therapies. Dermatol Online J. 2013 Jun.

Ingredients	Quantity (%) Rootness™ Energize formula	Quantity (%) placebo formula
Water	q.s	q.s
Ammonium Acryloyldimethyltaurate/ VP copolymer	0.80	0.80
Dicaprylyl ether	-	1.00
Rootness Energize (Dicaprylyl ether, *Luffa cylindrica* Root Extract)	1.00	-
Buffer solution (Citric acid, Sodium Citrate)	1.00	1.00
Phenoxyethanol, Methylparaben, Ethylparaben	1.10	1.10
Fragrance	0.10	0.10

Formula

contact

Amandine Werle[1], Charlotte Stricane[1]
Sandrine Delaunois[1], Benoit Mignard[1]
Alexandre Lapeyre[1], Agnès Guillaumin[2]
Natacha Rombaut[2], Jeremy Brouillot[2]
Mathilde Frechet[1], Hanane Chajra[1]

[1]Clariant Active Ingredients
195 route d'Espagne
31036 Toulouse Cedex 1, France

[2]Plant Advanced Technologies
PAT, 19, Avenue Forêt de Haye
54500 Vandoeuvre, France

Unleashed by the Power of AI

BASF's New Active Ingredient Protects Skin and Scalp against Silent Inflammation

P. Moussou, V. André-Frei, C. Kalem

abstract

Artificial Intelligence, or AI for short, is a cutting-edge digital technology that is generating a turbo-boost for modern research activities. The technology recently empowered BASF to discover innovative peptides derived from rice proteins by screening trillions of potential candidates. The result is called PeptAIde™* 4.0, a plant-based active ingredient that is clinically proven to protect skin and scalp against the damage caused by silent inflammation – including dry skin, irritations, discomfort and loss of firmness.

Introduction

Modern lifestyles are often complex, fast-paced and stressful – and expose people to a wide range of toxic agents that can accelerate the body's natural deterioration over time. As a result, people of all ages are becoming increasingly health-conscious, which is driving the rising popularity of self-care activities such as meditation, mindfulness and various approaches for healthy nutrition. Consumers are searching for ways to protect and maintain the youthful appearance of their hair, scalp and skin. In particular, they want solutions that can counteract the silent biochemical changes at the heart of the degenerative process.
These changes are caused when unhealthy lifestyles disturb one of the body's most important systems: inflammation. It involves responses that can be acute (short) or chronic (longer), and can occur in almost every part of the body. Typically, inflammation is a necessary and beneficial process that helps the body to heal and fight off infection by removing potential causes of injury from cells [1]. However, external factors such as a poor diet, air pollution or stress [2] can disrupt the process of inflammation and generate a negative effect on health.
This disrupted process, known as silent inflammation or inflammaging, fights against healthy cells and accelerates the body's degeneration – including causing deep damage to the hair, scalp and skin. While it is regarded as an unavoidable feature of aging [3, 4], it is also associated with several chronic illnesses including cardiovascular disease, Alzheimer's and diabetes [5, 6]. As a result, there is significant demand for solutions that can address and prevent the underlying mechanisms of silent inflammation.

Using AI to discover natural peptides that tackle silent inflammation

In recent years, peptides have become popular ingredients for cosmetic solutions that keep skin, scalp and hair healthy [7, 8]. Due to the popularity and relevant properties of these ingredients, a research team began exploring the potential for multifunctional peptides that are found in nature to fight against damage caused by silent inflammation.
The team faced some major challenges. This was primarily because cosmetic peptides are amino acid short chain oligomers that generally have between three and 20 amino acids – and the huge number of possible combinations of these amino acids means there is a very wide range of potential functions that peptides can perform. A random library of decapeptides with 10 amino acids, for example, would lead to 10^{12} possible sequences, while those with 20 amino acids would lead to 10^{26} possible sequences. Basically, there are just too many possible bioactive sequences to be screened by bioassays.
AI emerged as the solution to this problem because it is able to review and identify connections across massive volumes of data at rapid speed. The researchers created a predictive model that used four discrete data sources. First, public unstructured data from peer-reviewed scientific papers and patents. Second, public structured data from curated bioactivity annotations, biological pathways, structural annotations, as well as genomic and proteomic data relevant to immunomodulation. Third, peptidomic data amassed through LC-MS/MS (Liquid Chromatography with tandem mass spectrometry). And fourth, phenotypic data accrued from experimental screening of peptides in TNFα secretion in LPS (Lipopolysaccharide)-stimulated differentiated macrophages.

Plant-based biopeptides that protect the skin, hair and scalp

After screening trillions of data entries, the AI-based model identified four plant-based peptides, from 12 to 17 amino

Naomi, Anne and **Marion**
love to share fun, adventure – and values.

Care Creations®

BASF
We create chemistry

Their stories. Your products.
Our Plantacare®

The original APG® from BASF – celebrating 25 years in Europe!

Today's consumers want performance, safety and sustainability. Connection with nature is an integral part of people's lifestyles and values. Serve these needs and wishes with our **100% plant-based Plantacare® range** – now celebrating 25 years of outstanding quality and sustainability success in personal care in Europe.

Every story matters.
carecreations.basf.com

acids, with the highest potential to have a positive impact on silent inflammation. From this AI-based discovery, a plant-derived biopeptides product characterized by these four specific peptides was developed. BASF has now launched this innovative new naturally derived cosmetic ingredient under the name PeptAIde™ 4.0.

PeptAIde 4.0 is clinically proven to counteract the effects of silent inflammation – and keep skin and scalp healthy. A controlled enzymatic hydrolysis process was developed to unlock the four peptides from organic rice proteins (*Oryza sativa*) to produce PeptAIde 4.0 (INCI: Water (and) Hydrolyzed Rice Protein (and) Citric Acid). It is soluble in water and made of 99.6 percent material from natural origin according to ISO 16128. It is also compliant with the Cosmos standard for natural and organic cosmetics and compliant for use in China. On top of this, PeptAIde 4.0 is compatible with pH levels of between 3 and 8.5. It can be used in a large range of leave-on and rinse-off formulations for skin, scalp and hair care. And *in vitro*, *ex vivo* and *in vivo* tests have confirmed that it prevents dry skin, discomfort and loss of firmness, while also relieving irritations and discomfort from the scalp.

Proven to counteract the impact of silent inflammation

In vitro testing has clearly demonstrated that PeptAIde 4.0 is effective in counteracting the impact of silent inflammation on the skin, scalp and hair. Specifically, it counteracts the effects of tumor necrosis factor alpha (TNFα), which is a pro-inflammatory cytokine that functions as an inflammatory messenger. TNFα is proven to restrict the synthesis of the differentiation proteins involucrin and filaggrin by keratinocytes, as was also reported by Kim and coworkers [9]. When PeptAIde 4.0 was applied to a reconstructed epidermis (RE) at 0.01 percent dry weight (dw) before the addition of TNFα, it tripled the quantity of filaggrin when compared to control. At isodose, this effect is 2.4 times better than a comparative rice protein hydrolysate that does not contain the four targeted peptides. (**Fig. 1**)

By stimulating the synthesis of involucrin (data not shown) and filaggrin by keratinocytes in presence of TNFα, PeptAIde 4.0 counteracts the effect of silent inflammation on the expression of epidermal differentiation proteins. This can help to restore a normal epidermal differentiation and *stratum corneum* barrier – which prevents dryness and discomfort in the skin and scalp.

On top of this, in tubo testing showed that PeptAIde 4.0 reduces the activity of the degrading enzymes elastase and MMP12. It also stimulated the synthesis of Collagen I dermal fibers by fibroblasts and the growth of human hair papilla fibroblasts. In addition, tests on damaged hair strands showed a restoring effect in the hair cortex structure. All of these assays were compared to a rice protein hydrolysate that does not contain the four targeted peptides.

Taken together, these results show the potential for PeptAIde 4.0 to counteract damage to the skin, scalp and hair that is caused by silent inflammation.

In vivo tests show a positive impact on skin moisture

PeptAIde 4.0 has also demonstrated positive effects on skin and scalp during placebo-controlled clinical studies. A double-blind, split body (leg), placebo-controlled and randomized study was conducted on 24 women who have dry skin on their legs. They used the product in a body lotion formulated at 2 percent twice each day for four weeks to evaluate the effect on skin hydration and comfort compared to a placebo (body lotion vehicle alone). An additional evaluation was conducted after a regression period of one week without any product application.

PeptAIde 4.0 increased skin moisture after two weeks by 12 percent compared to the placebo lotion. After one month, 92 percent of participants stated that their skin felt soothed,

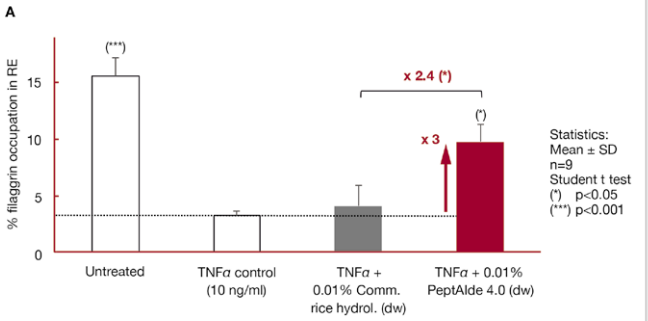


Fig. 1A Evaluation of the effect of PeptAIde 4.0 on filaggrin synthesis in a TNFα-treated reconstructed epidermis (RE). Comparative effect versus a rice protein hydrolysate lacking the four targeted peptides, at dry weight (dw) isodoses.

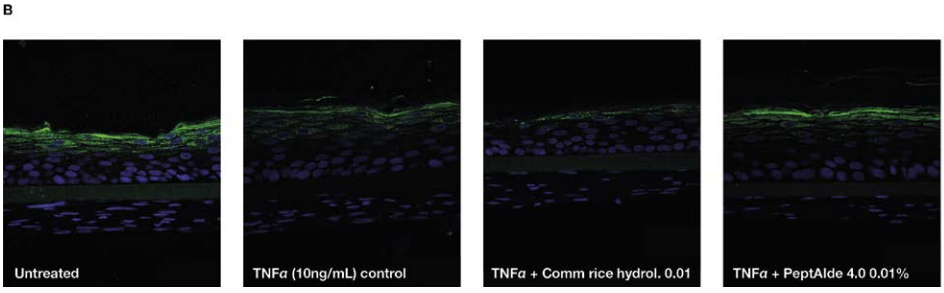


Fig. 1B Illustrative photograph by confocal microscopy of a histological section of an RE after immunostaining of filaggrin (in green) and cell nucleus DAPI counterstaining (blue). The results are expressed as dry weight (dw) for direct comparison: 0.01% dw corresponds to 0.05% PeptAIde 4.0.

smooth and nourished, while 88 percent stated that their skin felt more comfortable and less irritated. The results show that PeptAlde 4.0 increases skin moisture – and that this effect lasts even after product use has stopped. (**Fig. 2**)

Increased skin firmness confirmed *in vivo*

In another double-blind, split body (abdomen), placebo-controlled and randomized study, 34 female subjects aged from 40 to 65 years old applied a leave-on body lotion to their belly twice each day for 28 days. An additional evaluation was conducted after a regression period of one week without any product application.

After two weeks, skin firmness increased by 14 percent compared to the placebo lotion. In addition, 79 percent of participants stated that their skin was more supple, and 82 percent reported increased comfort after four weeks. These results demonstrate that PeptAlde 4.0 increases abdominal skin firmness – and that this effect lasts even after product use has been suspended. (**Fig. 3**)

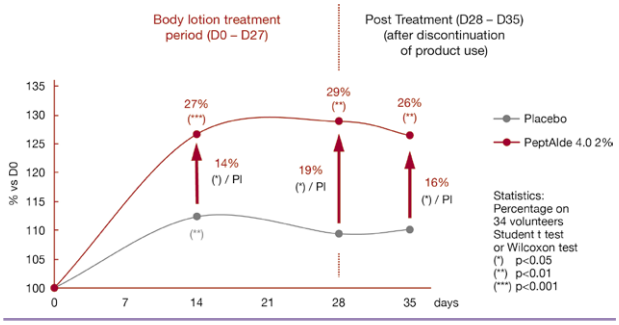


Fig. 3 Evolution in percentage of skin firmness (SkinFibrometer) with application of PeptAlde 4.0 at 2% and placebo over time versus baseline (D0).

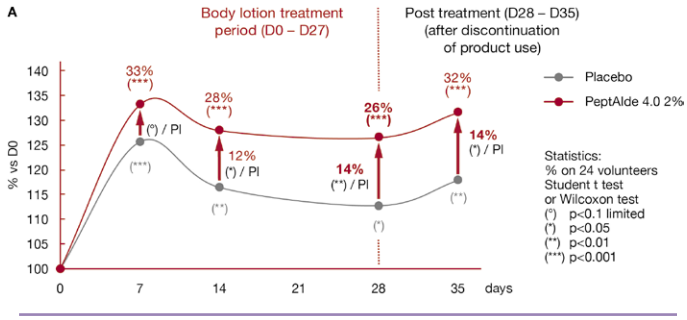


Fig. 2A Evolution in percentage of skin moisture (corneometer) with application of PeptAlde 4.0 at 2% in a body lotion and placebo over time versus baseline (D0).

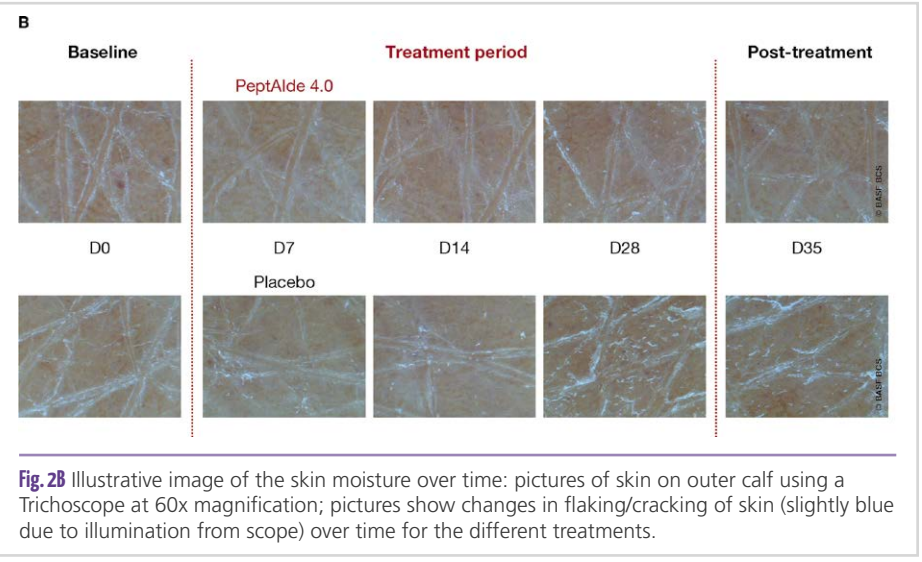


Fig. 2B Illustrative image of the skin moisture over time: pictures of skin on outer calf using a Trichoscope at 60x magnification; pictures show changes in flaking/cracking of skin (slightly blue due to illumination from scope) over time for the different treatments.

Decreasing redness and reducing the pH level in the scalp

A further double-blind, placebo-controlled and randomized clinical test was conducted to examine the impact of PeptAlde 4.0 on the scalp. 44 male and female participants with sensitive, irritated and scaly scalps used either a shampoo containing PeptAlde 4.0 at 2 percent or a placebo shampoo, which were applied three times each week for three weeks. An additional evaluation was conducted after a post-treatment period of one week, when the use of the test shampoo had been discontinued and the participants in both groups used a neutral shampoo instead.

Scalp redness decreased significantly and visibly for the participants who used the shampoo formula that included PeptAlde 4.0. The pH level of their scalp also decreased by 0.4 units, which is equivalent to the difference between a normal scalp and scalp with scaling and erythema problems [10]. The shampoo was mild to the scalp and did not show any disturbance to the normal level of the scalp's natural oil, sebum.

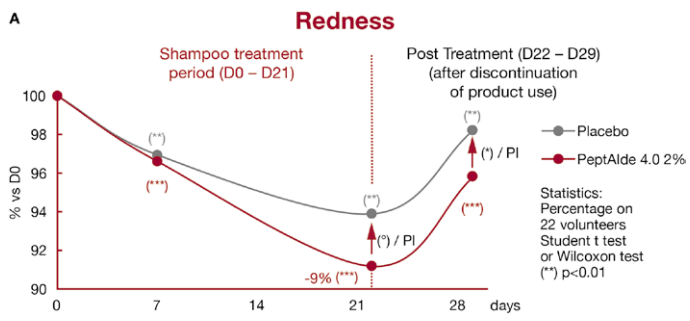


Fig. 4A Evolution in percentage of scalp redness (DermoGenius Ultra and image analysis) with use of a shampoo containing PeptAlde 4.0 at 2% and placebo over time versus baseline (D0).

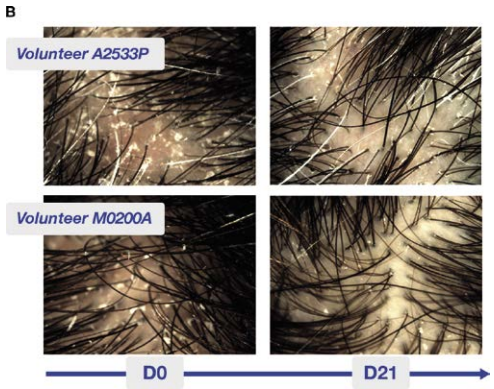


Fig. 4B Illustrative pictures of the scalp on two volunteers before and after three weeks of the shampoo containing 2% of PeptAlde 4.0.

These results show that the shampoo containing PeptAlde 4.0 is able to prevent damage and to restore a healthy scalp. (**Fig. 4**)

Conclusion

PeptAlde 4.0 is a naturally derived, multifunctional cosmetic ingredient for leave-on and rinse-off applications that is clinically proven to protect the skin, scalp and hair against damage caused by silent inflammation. Using AI and machine learning technology, researchers were able to screen trillions of data entries to identify four peptides with a high potential impact. A controlled enzymatic hydrolysis process was then conducted to unlock these peptides from organic rice proteins (*Oryza sativa*). The result is a next-generation plant-based biopeptides product that provides an answer to rising consumer demand for safe, natural and scientifically proven products for repairing the skin, scalp and hair.

Clinical testing has shown that BASF's PeptAlde 4.0 has a positive effect on the skin by increasing moisturization and making skin feel soothed, smooth and nourished after one month. It is also proven to increase skin firmness in the belly region and make the skin feel more supple. And the active ingredient has also demonstrated its efficacy related to decreasing scalp redness and lowering the pH level of the scalp – while also having a positive effect on conditions that support the repair of hair strands.

PeptAlde 4.0 gives consumers a versatile new weapon in the fight against damage caused by silent inflammation, and can have positive impact on keeping skin, scalp and hair healthy – at any age.

Formulations

Ageless firming Body Lotion
(SC-FR-20-BC-50892-03) (see **p. 50**)
Healthy Shampoo & Shower gel 2 in 1
(HB-FR-20-BC-50891-02) (see **p. 51**)

References

[1] *Medzhitov R.* Origin and physiological roles of inflammation. Nature 454, 428–435, 2008.

[2] *Kiecolt-Glaser JK.* Stress, food, and inflammation: psychoneuroimmunology and nutrition at the cutting edge. Psychosom. Med. 72: 365, 2010.

[3] *Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G.* Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 908: 244-254, 2000.

[4] *Franceschi C & Campisi J.* Chronic inflammation (Inflammaging) and its potential contribution to age-associated diseases. Journals Gerontol. - Ser. A Biol. Sci. Med. Sci. 69(Suppl 1): S4–9, 2014.

[5] *Murakami M & Hirano, T.* The molecular mechanisms of chronic inflammation development. Front. Immunol. 3: 1–2, 2012.

[6] *Holmes C et al.* Systemic inflammation and disease progression in Alzheimer disease. Neurology 73, 768–774, 2009.

[7] *Zhang L, Falla TJ.* Cosmeceuticals and peptides. Clin Dermatol. 27: 485–94, 2009.

[8] *Schagen SK.* Topical peptide treatments with effective anti-aging results. Cosmetics. 4, 16, 2017.

[9] *Kim IS, Kim MJ, Shin D-H, Son K-H, Park H-Y, Lee J-S.* Arazyme inhibits cytokine expression and upregulates skin barrier protein expression. Molecular Medicine Report. 8(2): 551-556, 2013.

[10] *Takagi Y, Takatoku H, Terazaki H, Nakamura T, Ishida K, Kitahara T.* The Scalp Has a Lower *Stratum Corneum* Function with a Lower Sensory Input than Other Areas of the Skin Evaluated by the Electrical Current Perception Threshold. Cosmetics. 2: 384-393, 2015.

contact

P. Moussou, PhD
V. André-Frei, PhD
C. Kalem

BASF Beauty Care Solutions France
Lyon | France

Corresponding author:
E-mail: Philippe.moussou@basf.com

Picture Credits: VIDI Studio/shutterstock.com

SOFW eVENTS

SOFW eVENT series

WeCONNECTING industries

You are passionated about personal care products?

Show your visitors that you are a professional in personal care and become an exhibitor at our events.

Don't miss your chance!

Book your virtual presence now!

eVENTS@sofw.com

www.SOFWeVENTS.com

Leveraging the Benefits of Probiotics for Oral Health with heat-treated Lactobacilli based on True Biotic Technology

T. Schmitter, J.T. Fischer, A. Maser, L. Schmidt, L. Garbe, C. Koch

Introduction

Modern lifestyle offering constant access to information has raised consumer awareness and demand for new, advanced technologies to improve their well-being and comfort. Consumers are increasingly looking for natural oral hygiene products that have the ability to protect their gums with high efficacy. According to Consumer Market Insight research, 63% of consumers expect toothpaste to sooth their sensitive gums and 69% of respondents liked the idea of using toothpaste with probiotics.
In reference to the WHO, probiotics are living microorganisms that, when administered in adequate amounts, confer a health benefit to the host [1]. The well-established concept in scientific literature and consumers' perception is that probiotics improve gut health [2]. Yet there are less reports about the benefits of probiotic microorganisms in oral care even though oral cavity hosts the second most diverse microbial community in the body [3]. A reason for this underrepresentation is that applying live bacteria in skin and oral care formulations represents considerable technical challenges with regard to handling, storage and safety of the respective finished market products. To circumvent these hurdles, Symrise developed in 2019 a probiotic-derived product combining multiple skin health benefits of a probiotic with the advantages of a classical cosmetic ingredient in terms of ease of use in various skin care applications. Today, based on the same True Biotic technology, Symrise is broadening its portfolio by introducing a new ingredient dedicated to oral care applications.
Working in close collaboration with the probiotics specialist Probi AB in Sweden, Symrise identified a specific Lactobacillus strain for oral care applications: *Lactobacillus plantarum* HEAL19.

Materials & Methods

Microorganism and True Biotic technology

Lactobacillus plantarum HEAL19 (supplied by Probi AB) is mildly heat-treated and spray dried following our True Biotic technology, where specifically selected processing conditions ensure that the final material consists of non-living probiotic bacteria with structurally intact hulls. Acacia Senegal Gum, a tooth friendly biopolymer that is widely used in food applications, serves as final carrier material. Performance of all experiments presented below was with the heat-treated material without any additional carrier material, except the tests regarding cell integrity and stability in formulation.

Components Mouthwash	Components Toothpaste
Aqua	Aqua
Sorbitol (70%)	Sorbitol (70%)
Xanthan Gum	Silica
Sodium Saccharin	PEG-32
Sodium Benzoate	Cellulose Gum
Sodium Fluoride	Sodium Lauryl Sulfate
Lactic Acid (90%)	Titanium Dioxide
PEG-40 Hydrogenated Castor Oil	Sodium Monofluorophosphate
Propylene Glycol	Methylparaben
OPTAMINT® Flavor	Trisodium Phosphate
	Saccharin
	OPTAMINT® Flavor
Processed material: Acacia Senegal Gum, Lactobacillus Ferment	Processed material: Acacia Senegal Gum, Lactobacillus Ferment

Tab. 1 Oral Care Formulations

Oral care formulations: toothpaste and mouthwash

Processed material was incorporated in two oral care formulations: toothpaste and mouthwash (formulation details in **Tab. 1**).
Due to its optimized product characteristics – the processed material is a non-hygroscopic nearly white powder, processable at temperatures up to 80°C, tolerates high shear mixing and is stable over a broad pH range in semi-solid and liquid formulations, the

SymReboot™ OC

True Biotic

Sustaining oral cavity's instinctive defenses

Based on a novel patented technology, Symrise introduces its new processed probiotic.
The bacterium membrane is kept intact, thus delivering its full specific benefits to the oral cavity.

#MICROBIOTA PATHWAYS REGULATION
#SOOTHING
#ORAL MUCOSA BARRIER STRENGTHENING

COSMETIC INGREDIENTS BY SYMRISE
always inspiring more ...

www.symselect.com I www.symrise.com
Follow us on Instagram @symrise.ci

material is easy to handle and can be incorporated in any kind of formulation without the use of specific stabilizers, any incompatibilities are not known. The toothpaste was prepared in a Stephan Cutter (Universal Machine UMC5, Stephan Food Service Equipment GmbH, Hameln, Germany) under partial vacuum to avoid formation of air pockets during mixing. The processed material was conveniently added in the last production step under intensive stirring. Preparation of the mouthwash was completed by adding the single ingredients stepwise to the aqueous mixture. In accordance with the toothpaste preparation, the processed material was incorporated as a last production step and intensively stirred until uniform distribution.

Assessment of cell integrity by fluorescence microscopy

Solutions of viable lyophilized bacteria, heat-treated *L. plantarum* HEAL19 and the final mouthwash formulation including our product were diluted with 1 x PBS (Sigma-Aldrich). Samples were stained with either SynaptoRed C2 (FM4-64, Biotium, C_F=10µM), Hoechst 34580 (ThermoFisher Scientific, C_F= 2µM) or double stained for 20 minutes at room temperature in the dark. Thereafter, samples were washed one time with 1 x PBS and incubated on cover slips (ThermoFisher Scientific) for 3 hours at room temperature in the dark. After air-drying the cover slips were mounted with SlowFade Diamond solution (ThermoFisher Scientific, ProLong Glass Antifade Mountant) on microscopic slides and analyzed by an Olympus BX43 microscope with a mercury arc fluorescence illumination light source (U-HGLGPS, Olympus GmbH).

Barrier strengthening

The effect of heat-treated *L. plantarum* HEAL19 on barrier integrity was investigated based on filaggrin-release in 3D skin models *in vitro* as well as on human skin explants *ex vivo* (data not shown).
The 3D human skin models derived from human epidermal keratinocyte progenitors (HEPKp, CELLnTEC) were treated systemically with test substances in CnT-PR-3D medium for up to eight days. Filaggrin levels were quantified in the supernatant of the treated and control samples using ELISA technique according to manufacturer`s instructions (Cloud-Clone Corp.). The viability of the skin models after the treatment was evaluated by MTT-tests (data not shown).

Pathogen defense

Anti-microbial peptide (AMP) expression levels were determined *in vitro* with human reconstructed epidermal 3D models (data not shown) as well as *ex vivo* using human skin explants. Skin explants were treated topically with a hydro dispersion gel containing heat-treated *L. plantarum* HEAL19 for up to 6 days. For the gene expression analysis, total RNA was extracted from skin biopsies by using RNeasy mini kit for fibrous tissues (Qiagen) following manufacturer's instructions. Following quantification, 1 µg of total RNA was retro-transcribed by using High-Capacity RNA-to-cDNA™ Kit (ThermoFisher Scientific). The produced cDNA was then used to perform the gene expression analysis using TaqMan® Array Fast Plates and TaqMan® Fast Universal PCR Master Mix (ThermoFisher Scientific). Plates were coated with the following targets: S100 calcium binding protein A8 Hs00374263_m1, S100 calcium binding protein A9 Hs00610058_m1 and ribonuclease A family member 7 Hs00261482_m1. Analysis was done by 2-ΔCT method. Gene expression was normalized on the housekeeping gene hypoxanthine phosphoribosyltransferase 1 Hs99999909_m1. The Real-time PCR experiments were performed on QuantStudio 3 Real-Time PCR System (ThermoFisher Scientific).

Assessment of soothing in primary human monocytes

Human-derived primary monocytes were extracted from blood cells of healthy blood donors (University Hospital of Freiburg, Germany) by a gradient protocol described elsewhere [4]. Cells were seeded in 24 well-plates at 2.2 x 10^6 cells per well and stimulated with pyrogenic LPS (10 ng/ml, Salmonella enterica serotype typhimurium SL1181, Sigma) for 30 minutes, followed by co-incubation with various dosages of heat-treated *L. plantarum* HEAL19 for 24 hours. Thereafter, supernatants were harvested and inflammatory parameters assessed by enzyme immunoassays according to manufacturer's instructions. Parallel samples assessed cellular viability by alamarBlue™ staining (ThermoFisher Scientific).

Impact on oral biofilm microbiome

The impact of the material on human oral biofilms was investigated in a representative *ex vivo* biofilm model [5]. Complex oral biofilms derived from pooled human saliva were grown on hydroxyapatite-coated pegs for 14 days. Cells were left untreated at 37°C and 5% CO_2 for 7 days with change of growth medium every 3.5 days. After 7 days of growth, biofilms were treated twice a day with heat-treated *L. plantarum* HEAL19 (biomass equivalent of 1 x 10^8 CFU/ml), a medium control or a positive control for 7 days. Treatment occurred for 1 minute on a shaker, followed by 30 seconds wash in 1 x PBS. Biofilm composition was analyzed by partial 16S rRNA gene pyrosequencing on a Roche 454 sequencer followed by analysis using the mothur pipeline. Overall, 486,261 16S rDNA sequences were obtained. Sequences were clustered into operational taxonomic units (OTU) at 1.5% genetic distance and identified using a Naive Bayesian classifier with the Human Oral Microbiome database (homd.org). Relative abundance on genus-level for *L. plantarum* HEAL19, treated versus untreated control, was analyzed based on total reads of six biological replicates.

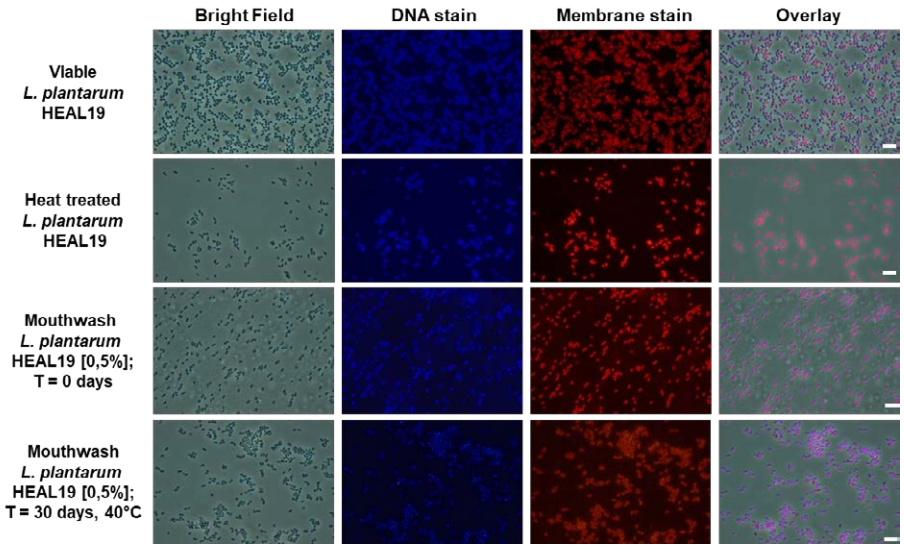


Fig. 1 *L. plantarum* HEAL19 mildly processed by our True Biotic technology and applied in oral care mouthwash does not alter the structural integrity of the bacterial cell hull. Samples were stained with Hoechst 34580 and SynaptoRed C2 to illuminate bacterial DNA and lipid parts of the membrane respectively. (Pictures were taken with Olympus RX at a 1000x magnification. Bar represents 5 µM).

Results & Discussion

Product characteristics and integrity of cell structure

The final product is a non-hygroscopic, nearly white powder that, in contrast to most living probiotics, can be stored under standard conditions and easily be incorporated into different formulations (see Materials & Methods). It is commercialized as OPTABIOTICS® 24 and SymReboot™ OC by Symrise AG.
Fluorescence microscopy was applied to visualize the quality of the processed material and its stability in the final formulated products. We confirmed that the True Biotic production technology, i.e. mild heat-treatment and spray drying, does not change the microscopic phenotypical appearance of the material when compared to structurally intact, viable bacteria (**Fig. 1**). Even 30 days at 40°C in an oral care formulation, like a detergent-containing mouthwash, caused no visible disruption of the bacterial cell structure as demonstrated after staining (**Fig. 1**).

Barrier strengthening and pathogen defense

Barrier strengthening benefits were exemplarily investigated *in vitro* on human skin models and *ex vivo* on skin explants. A significant and dose-dependent filaggrin induction was found after treatment of the *in vitro* 3D models and confirmed in *ex vivo* experiments.
An applied concentration of 0.05% *L. plantarum* HEAL19 led to an increase of filaggrin secretion of 115% compared to medium control (**Fig. 2**) after eight days systemic treatment. The effect on filaggrin biosynthesis was further verified by histochemical staining in *ex vivo* experiments (data not shown).
The experiments on AMP induction revealed that *L. plantarum* HEAL19 interacts with the human host cells and enhances host's pathogen defense. A dose-dependent up-regulation of antimicrobial peptides expression was found in *ex vivo* experiments. Two genes of the Calprotectin complex encoding the S100A8 and S100A9 proteins as well as the RNAse7 gene were induced up to 8-fold compared to the untreated control (**Fig. 3**).

Modulation of inflammatory response

Inflammation of the gingival epithelium is a key event in the progression of periodontal disease leading to chronic periodontitis when left untreated [6]. Formation of plaque and the colonization of disease-associated bacteria evoke a pyrogenic LPS-induced immune response of host cells resulting in local migration of immune cells to the focus of infection. Using primary human cells from healthy blood donors, we mimic such an inflammatory event, stimulating human monocytes with bacterial-derived, pyrogenic lipopolysaccharide molecules. Clearly, in the absence of any cytotoxic impact (**Fig. 4b**), treatment of stimulated cells with *L. plantarum*

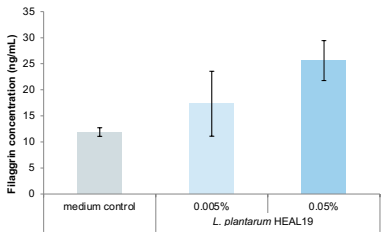


Fig. 2 Filaggrin concentration released from human 3D skin models after eight days systemic treatment with heat-treated *L. plantarum* HEAL19.

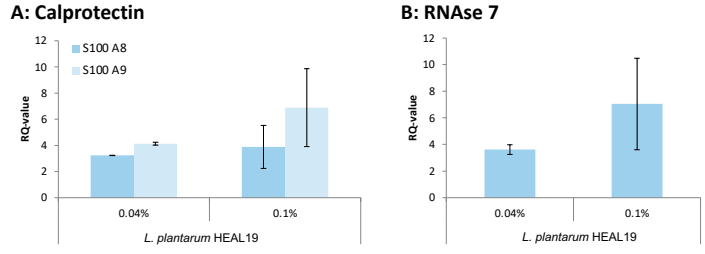


Fig. 3 Induction of AMP gene expressions in *ex vivo* human skin explants after topical treatment with *L. plantarum* HEAL19 in a hydro dispersion gel.
A: S100A8 and S100A9 (Calprotectin complex); B: RNAse7

HEAL19 resulted in a dose-dependent decrease of Interleukin-1ß; one of the major cytokines in orchestrating the immune defense (**Fig. 4a**), thus clearly representing successful soothing of the cells.

Biofilm modulation

The biofilms grown in the *ex vivo* oral biofilm model mirror the diversity and richness of the oral cavity with more than 250 different species. In essence, the microbial composition of the biofilms resembles that of dental plaque with *Streptococcus, Veillonella* and *Prevotella* as predominant genera [7]. The interventional treatment with *L. plantarum* HEAL19 differentially affects the relative abundance of predominant genera in the biofilm model (**Fig. 5**) but not the overall biodiversity. While genera associated with periodontal health, as *Corynebacterium* and *Neisseria* [8] increase their abundance compared to the control biofilms (**Fig. 5a**), other genera such as *Bacteroides, Dialister, Fusobacterium, Prevotella, Pyramidobacter* and *Veillonella* are reduced in their abundance (**Fig. 5b**). Remarkably, the latter gram-negative anaerobes have been associated with the onset of gingivitis in human clinical studies of experimental gingivitis [9]. In conclusion, the addition of *L. plantarum* HEAL19 has a positive microbiome modulating effect.

Conclusion

According to the described efficacies, the mildly heat-treated *Lactobacillus plantarum* HEAL19 based product has the following benefits:

- Enhancing oral cavity's defense against pathogens by modulation of AMP (*in vitro, ex vivo*).
- Strengthening the oral mucosa barrier by filaggrin production *(in vitro, ex vivo*)
- Soothing the gums (*ex vivo*)
- Supporting a healthy oral microbiome by limiting the abundance of harmful bacterial species and promoting health-associated ones (*ex vivo*)

In conclusion, our newly developed product is delivering the advantages and properties of a probiotic product without the disadvantages (e.g. handling, storage) of a living material. The product is an easy to handle ingredient that delivers multiple oral care benefits.

Acknowledgements

We are grateful to *Prof. William Wade* (King's College,London, UK, Centre for Host-Microbiome Interactions) for establishing and conducting the oral biofilm experiments. We would like to thank *Dr. Bernd Fiebich* (Vivacell) for ongoing support. We also would like to thank *MSc Christine Koenig* for expert technical assistance with fluorescence microscopy. Finally, we want to thank Probi AB for their great contribution in the product development.

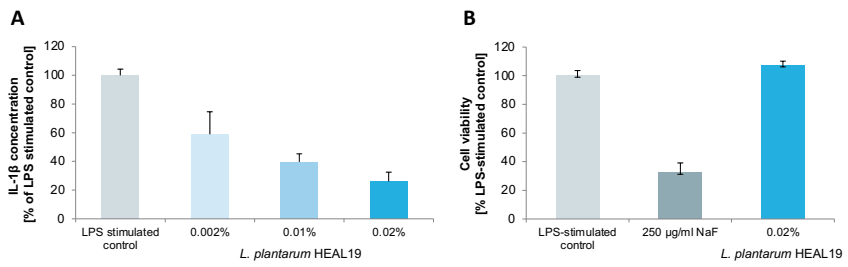


Fig. 4 Dose-dependent soothing effect of *L. plantarum* HEAL19 on LPS-stimulated monocytes. The stimulated monocytes treated with indicated doses [w/w] for 24 hours. IL-1ß level as detected by ELISA calculated in reference to untreated control (A). No cytotoxic effect detected of the analyte at highest dose by Alamar Blue assay (B). Graphs show a single experiment conducted with three biological replicates.

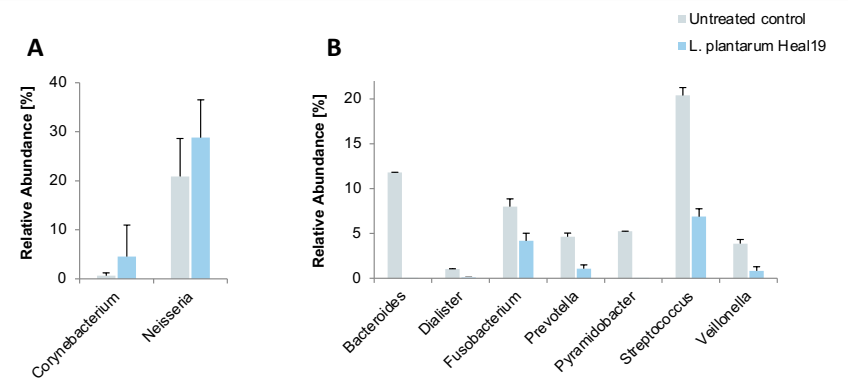


Fig. 5 Relative abundancies of predominant genera in *ex vivo* oral biofilm model treated with *L. plantarum* HEAL19. (A) Increase in relative abundance of health-associated *Corynebacterium* and *Neisseria* genera due to treatment with *L. plantarum* HEAL19 compared to untreated control. (B) Decrease in relative abundance of oral disease associated genera in treated versus untreated samples. Graphs show relative abundance of operational taxonomic units on genera level after 16S rRNA sequencing. Single experiment done with six biofilm replicates.

References

[1] FAO/WHO definition of probiotics (2001)

[2] *Reid et al.,* Nat Rev Microbiol. 2011; 9(1):27-38 3

[3] *Kilian et al.,* Br Dent J (2016); 221,657–666

[4] *Schmitter, T., et al., Ex-vivo* anti-inflammatory effects of probiotics for periodontal health. J Oral Microbiol, 2018. 10(1): p. 1502027.

[5] *Kistler, J.O., M. Pesaro,* and *W.G. Wade,* Development and pyrosequencing analysis of an *in-vitro* oral biofilm model. BMC Microbiol, 2015. 15: p. 24.

[6] *Schatzle, M., et al.*, Clinical course of chronic periodontitis. I. Role of gingivitis. J Clin Periodontol, 2003. 30(10): p. 887-901.

[7] *Aas, J.A., et al.,* Defining the normal bacterial flora of the oral cavity. J Clin Microbiol, 2005. 43(11): p. 5721-32.

[8] *Kistler, J.O., et al.,* Bacterial community development in experimental gingivitis. PLoS One, 2013. 8(8): p. e71227.

[9] *Diaz, P.I., et al.,* Molecular characterization of subject-specific oral microflora during initial colonization of enamel. Appl Environ Microbiol, 2006. 72(4): p. 2837-48.

authors

Tim Schmitter[1], Jörg Thilo Fischer[1], Andres Maser[1], Léa Schmidt[2], Lisa Garbe[1], Christin Koch[1]

[1]Symrise AG, Mühlenfeldstraße 1, Holzminden, Germany
[2]Symrise SAS, 13-17 rue Mozart, Clichy, France

13–15 OCTOBER 2021

SEPAWA® CONGRESS

Why should you visit the SEPAWA® CONGRESS VIRTUAL?

- **120 lectures** on the subjects of washing, cleaning, cosmetics and fragrance
- Experience our **evolved plattform**
- **Chat** with our **experts from the industry** and talk real business
- Make **new contacts** with our Networking Carrousel
- Use our **SEPAWA® CONGRESS App** to request meetings and many more possibilities

MEET YOUR BUSINESS FROM WHEREVER YOU ARE

www.sepawa-congress.com

#sepawacongress
#welovesepawa

Effects of Additives on the Phase Behavior of an EO/PO-Copolymer

J. Birnbach, J. Esteban, T. Hellweg, P. Schmiedel

abstract

Certain EO/PO-triblock polymers can form densely packed spherical micelles and cubic liquid crystalline phases at high concentrations. Usually these are highly elastic and inherently stable gels. This article deals with the effect of various additives on the phase behavior of an EO/PO-triblock polymer, in particular on the existence range of the cubic phase. The effects were characterized by means of rheology, zeta potential measurement, DOSY, and SAXS. It was found that some additives e.g. ionic surfactants decreased the existence range of the cubic phase, whereas others e.g. saccharides showed the reverse effect. The predominant mechanisms for these opposite effects might be different for various additives. However, certain regularities were identified probably due to three different interfering mechanisms. One mechanism is related to additives which can change the polymer-water interface, e.g. n-butanol. Other additives might change the hydration shell of the EO-PO-polymers, e.g. maltose. Furthermore, complexation processes could occur, e.g. in case of surfactants, which decrease the hydrophobicity of the PPO-part.

Introduction

Poloxamers or Pluronics® are triblock copolymers consisting of a block of poly(propylene oxide) (PPO) which is enclosed by two blocks of poly(ethylene oxide) (PEO) at both sides [1]. They are available with different block lengths of propyleneoxide (PO) and ethylenoxide (EO) units. Poloxamers form various lyotropic liquid criystalline phases which is why they are used in many industrial applications, e.g. in creams and ointments [2]–[4]. For intance, the EO/PO-polymer $(EO)_{74}(PO)_{30}(EO)_{74}$ reported in this study forms a cubic phase which is a highly elastic gel - sometimes called "ringing gel". However, it is usually necessary to add more components to the liquid crystalline phase formed by a poloxamer because further active ingredients are required in commercial applications. For example, ionic surfactants are added in paints, coatings, laundry detergents, cosmetic products and pharmaceutical formulations. Further examples are drug delivery systems which are a promising development of the past years [5]. The cubic phase formed by EO/PO-polymers can be used for this purpose which requires the addition of further active ingredients. By adding these ingredients, the phase behavior of the EO/PO-polymer changes. This may lead to the desintigration of the liquid crystalline phase which is crucial for the correct functionality and viscosity of the system. Therefore, it is necessary to investigate the impact of additives on the phase behavior. The effect of single additives as ionic surfactants [3],[6]–[11], salts [3],[12]–[19], short chain alcohols [20]–[23], and polyols [2],[7],[24] has been discussed extensivly, but most publications just focus on one addditive. In order to obtain a comprehensive picture of how additives change the phase behavior of a poloxamer and to predicit the effects of any additive, it is useful to consider all kinds of additives simultaneously. Not much is known about synergetic or overlaying effects and mechanisms of the different ingredients in multicomponent systems. In this paper we present a comprehensive study of the effect caused by various types of additives like surfactants, electrolytes and solvents on the phase behaior of $(EO)_{74}(PO)_{30}(EO)_{74}$. Structural changes are detected by means of rheology, small-angle x-ray scattering (SAXS), zeta potential determination and diffusion ordered spectroscopy (DOSY).

Materials and Methods

Materials

The examined EO/PO-polymer was Pluronic PE6800 purchased from BASF SE, LAS (Disponil LDBS55) from BASF SE (57% active substance), sodium dodecyl sulfate (SDS) from VWR Chemicals, 1,2-propylene glycol from Merck KGaA (>99.5% pure), glycerol from Merck KGaA (>99% pure), D(-)-fructose from Carl Roth GmbH + Co. KG (>99.5% pure), D(+)-maltose

monohydrate from Carl Roth GmbH + Co. KG (>95% pure), D-glucose anhydrous from Thermo Fisher Scientific Inc., sodium chloride from Merck KGaA., ammonium acetate from Thermo Fisher Scientific Inc., calcium chloride (>97% pure) from Appli Chem, potassium iodide from VWR Chemicals, sodium bromide (>99% pure) from Merck KGaA, magnesium chloride hexahydrate from Sigma-Aldrich, aluminum acetate hexahydrate (>95% pure) from Carl Roth GmbH + Co. KG and sodium acetate (99% pure) from Alfa Aesar. Ammonium chloride, sodium hydrogen carbonate, magnesium acetate tetrahydrate, n-butanol and sodium carbonate were received through internal resources. Technical substances have been used as supplied without further purification.

Preparation of the Gel-like Samples

From the rheological point of view the prepared samples behave gel-like. The preparation of these gels is described below: after the EO/PO-polymer was melted at 80°C, surfactants and solvents were added while stirring in a hot water bath. As they were dissolved, water and salt solution were mixed to the blend. This often led to an increase of the viscosity of the blend because of the phase transition from the isotropic to the cubic phase. Therefore, it was tried to liquefy the gel in an ice-bath. If the gel did not liquefy, it was necessary to stir it vigorously with a spatula. For complete homogenization and elimination of air bubbles, the gels were centrifuged at 3000 rpm for 1 h in the centrifuge Megafuge 2.0R from Heraeus Instruments. All gels were kept at least for 24 h for reaching an equilibrium before they were measured.

Methods

The gels were then characterized by rheology. The rheological measurements were carried out using the TA-Instruments AR-G2 Rheometer. The geometry was a steel cone with a diameter of 20 mm and a cone angle of 4°. Stress sweeps were performed at a frequency of 1 Hz from 0,01 Pa to 1000 Pa at 20°C. Within the existence range of the cubic phase the formulations are highly elastic: the storage modulus G′ is significantly higher than the loss modulus G″ which is why the tan δ = G″/G′ is small. If the tan δ becomes larger, the viscous properties increase and the phase boundary of the isotropic phase and the cubic phase becomes closer. As described subsequently, the tan δ indicates the distance to the phase boundary within the cubic phase. By comparing the tan δ at 100 Pa, different compositions could be related. SAXS measurements were performed with the Xeuss WAXS/SAXS 1.0 system from XENOCS, France at 25°C. DOSY was employed to analyze association phenomena. It was measured using an OneNMR probe of the apparatus Agilent DD2 600 MHz. The length of the gradient was 3 ms and the time of diffusion was 500 ms. The zeta potential was measured thrice at 25°C using the Zetasizer Nano ZS from Malvern Instruments.

Results and Discussion

Concentration of the EO/PO-polymer

Fig. 1 displays the tan δ as a function of the concentration of the polymer. The higher the content of the EO/PO-polymer, the lower is the tan δ. Therefore, a low tan δ indicates that the system has larger distance to the phase boundary between the cubic and the isotropic phase in the phase diagram. The phase transition occurs at a polymer concentration of 30 wt%. By means of SAXS measurements, it was found that a body centered cubic phase is formed. **Fig. 1** displays the correlation between the tan δ and the intensity of the SAXS peak with the Miller indices (100). With increasing concentration of the EO/PO-polymer, the SAXS peaks become more intense and sharper. Thus, the decreasing tan δ and the more intense SAXS peaks could be interpreted as a long-ranged order of the cubic phase. The higher the order, the more elastic is the cubic phase (low tan δ) and the more intense are the SAXS-peaks. Therefore, the relatively easy measurement of the tan δ can be regarded as an efficient tool for the characterization of the degree of order of the cubic phases and will be used for studying the influence of additives further on.

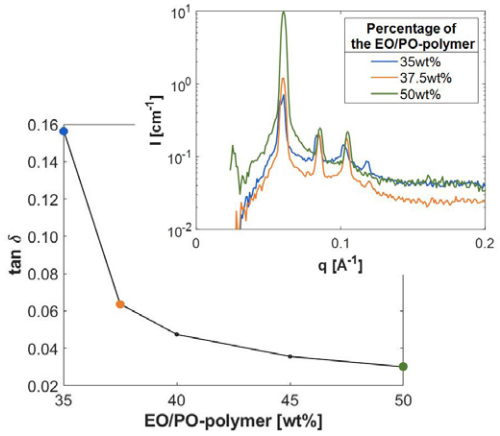


Fig. 1 The rheological tan δ is displayed as a function of the concentration of the EO/PO-polymer. The decreasing tan δ correlates with the increase of intensity of the peak with the Miller index (100) of SAXS measurements.

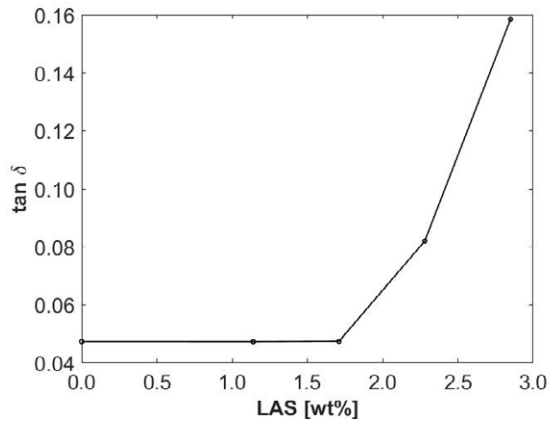


Fig. 2 Tan δ as a function of the concentration of LAS.

Surfactants

As surfactants are a common ingredient for commercial products, their influence on the cubic phase was analysed. In previous studies, we found that surfactants destabilize the cubic phase. This destabilising effect was found to correlate with the ionic strength of the surfactant. To get a deeper insight into this effect in case of ionic surfactants, the tan δ was analyzed at different concentrations of LAS. **Fig. 2** depicts that the tan δ remains constant up to 1.8 wt% LAS, but at higher concentrations the tan δ increases very strongly. Additionally, the diffusion coefficients of pure SDS and of SDS with the EO/PO-polymer in the cubic phase were determined by DOSY-NMR (**Fig. 3**). Free SDS molecules have a diffusion coefficient of $4 \cdot 10^{-10}$ m²/s at 0.1 wt% which is a concentration below the critical micelle concentration. In contrast, the diffusion coefficients of SDS and the EO/PO-polymer within the cubic phase have the similar value of lower than $0.2 \cdot 10^{-10}$ m²/s. This indicates that the SDS associates with the polymer. Additionally, the zeta potential was measured to study the surface electric potential of the (mixed) polymer micelles. The zeta potential of the pure EO/PO-polymer solution is close to zero, whereas the zeta-potential of the pure solution of LAS has a higher negative zeta potential (-45 mV) due to its anionic charge. The mixture of the polymer and LAS shows a

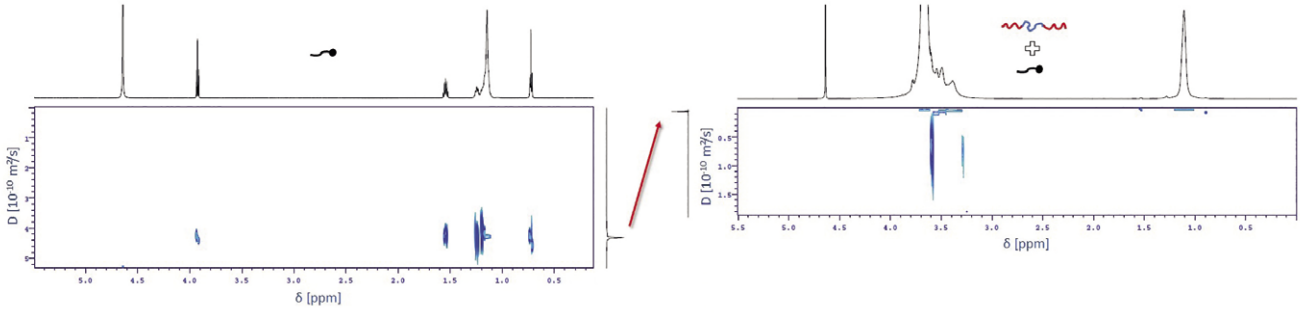


Fig. 3 DOSY-PFG-NMR-spectra displaying the diffusion coefficients of free SDS at 0.1 wt% (left) and of the mixture of 40 wt% EO/PO-polymer and 0.1 wt% SDS.

zeta-potential of the pure substances (-5 mV) which lays between the values of the two components. This result confirms the assumption of an association of the ionic surfactant with the polymer micelle.

Hence, the following dominating mechanism is proposed: if adding surfactants to the solution of the EO/PO-polymer, the surfactants bind to the hydrophobic PPO-part of the polymer. As a result, the hydrophobic part becomes charged. This leads to an increase of the solubility and to a decrease of the polymer´s amphiphilic character [7]. Due to this decreased amphiphilic character, the micelle formation is depressed if the surfactant concentration increases. Thus, the mean particle size decreases and small aggregates are formed consisting of few polymer molecules [2],[5],[7]. The volume fraction decreases resulting in the disintegration of the cubic phase [9]. This might be accelerated by increased electrostatic interactions of the charged micelles. The mixed micelles repel each other resulting in the hindrance of coagulation. At high surfactant concentrations, the PPO-part of the polymer is reported to be saturated with a specific amount of ionic surfactant molecules. The remaining surfactant molecules form own micelles and the PPO-part is fully stretched (**Fig. 6**) [5],[6].

Salts

The addition of salts to the cubic phase of an EO/PO-polymer appeared to extend the existence range of the cubic phase and to decrease the cloud point. For analyzing this effect, different salts were added at the same molar concentration of 7.8 mmol (**Fig. 4**). Some anions and cations show a stronger effect on the cubic phase than others. In literature, the order of the ability of an ion to trigger phase transitions of an EO/PO-polymer has been correlated with the Hofmeister series [3],[11]. However, our obtained results are not in satisfactory accordance with the Hofmeister series. Nonetheless, the obtained series of salts correlates with the ion´s enthalpy of hydration. It was found that increasing hydration enthalpy is linked to the ability of the ions to increase the existence range of the cubic phase.

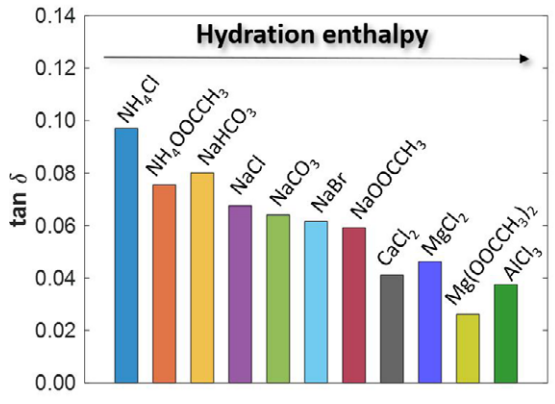


Fig. 4 Comparison of the effect of 7.8 mmol different salts on the cubic phase.

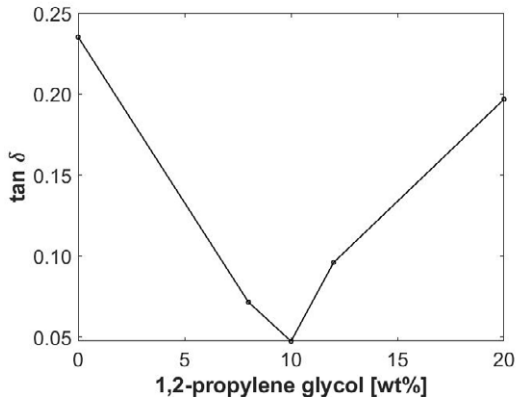


Fig. 5 The tan δ is displayed as a function of the concentration of 1,2-propylene glycol. The minimum indicates two different overlaying mechanisms.

Therefore, the following mechanism is suggested when adding salts to a cold aqueous solution of an EO/PO-polymer. Ions firstly dehydrate the PPO-block of the polymer due to the higher hydration enthalpy of the ion than the one of the polymer. This causes an increase of the hydrophobicity of the PPO-part leading to an enhancement of the polymer´s amphiphilic character. In this way, the formation of micelles is promoted. If the concentration of the ions increases further, the PEO-part also dehydrates. The micelle´s hydration shell decreases, and the tails of the PEO-part can interact with each other. This leads to an attractive force of the micelles and to an aggregation as sticky hard spheres. On a further increase of the salt concentration, the hydration water of the PEO-part vanishes, and phase separation occurs (**Fig. 6**).

Polyols

The influence of different polyols on the cubic structure and on its rheology was investigated. In the cases of glycerol and fructose the tan δ decreases up to a concentration of 20 wt%. Thus, glycerol and fructose increase the existence range of the cubic phase up to 20 wt%. However, in the case of other polyols as 1,2-propylene glycol, glucose, and maltose a minimum appears shown for example in **Fig. 5**. The tan δ firstly decreases until reaching a minimum and subsequently increases with an increasing content of the additive. This implies the superposition of two opposite effects. The stabilizing effect for the cubic phase may be similar to the effect of salts, glycerol, and fructose. However, other mechanisms can occur besides the dehydration effect. For example, the addition of substances can change the interfacial tension between the hydrophobic PPO-part and the aqueous phase. If the interfacial tension is reduced, the driving force for micellization is lowered. In literature, it has already been reported that 1,2 propylene glycol acts as a co-surfactant [7]. As it may be located at the interface between the PPO- and the PEO-part, it links the PPO-part to water molecules (**Fig. 6**). Comparing the decrease of the interfacial tension of 1,2-propylene glycol and glycerol as a function of the content, 1,2-propylene glycol leads to a steeper drop than glycerol. For example, the surface tension of the 1,2-propylene glycol solution has already dropped to approximately 59.5 mN/m at 10 wt% while glycerol still has a surface tension of 72.5 mN/m at the same concentration. It could be assumed that the behavior at the PPO/water interface is similar. This might be the reason for the increase of the tan δ. This effect can be better observed in case of n-butanol. If adding n-butanol to the cubic structure, its influence is even more significant. An aqueous solution of 5 wt% n-butanol only has a surface tension of approx. 30 mN/m. Indeed, the addition of 5 wt% n-butanol leads to the transition from the cubic phase to the isotropic phase. This outcome is not in agreement with other results found in literature which reports that butanol facilitates the micelle formation [20].

Conclusion

In this article, the effects of various additives on the cubic phase formed by the EO/PO-polymer $(EO)_{74}(PO)_{30}(EO)_{74}$ are described, interpreted, reviewed, and compared with literature. It was found that additives can extend or shrink the existence range of the cubic phase, respectively. A high poly-

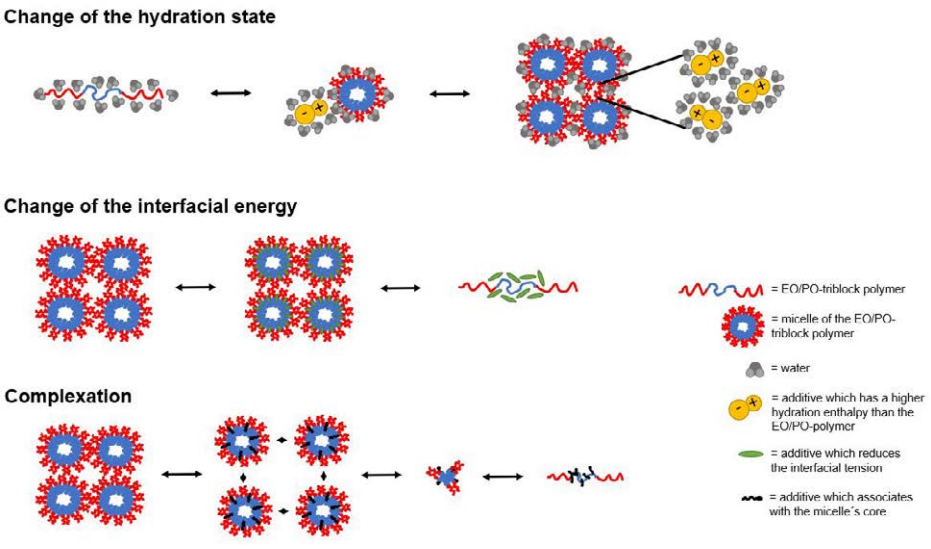


Fig. 6 schematic representation of the proposed mechanisms which lead to an increased existence range of the cubic phase (if adding an additive which has a higher hydration enthalpy than the polymer, top) or decrease the existence range (if adding additives which reduce the interfacial tension or associate with the micelle´s core, middle and bottom).

mer concentration, a large amount of well hydrated ions, water binding substances as glycerol and fructose stabilized the cubic phase at any concentration. In contrast glucose, maltose and 1,2-propylene glycol also showed a stabilizing effect at low concentrations, but a destabilizing effect at higher concentrations. Surfactants and n-butanol reduced the existence range of the cubic phase at all concentrations. The stabilizing effects on the cubic phase are based on the additive´s ability to dehydrate the polymer and simultaneously their low impact on the interfacial tension. Due to the dehydration of the PPO-part, the amphiphilicity of the EO/PO-polymer increases which favors the formation of micelles. If the PEO-part also begins to dehydrate, the hydration shell of the polymer micelle decreases. This leads to enhanced interactions between the PEO-parts and the sticky hard sphere potential rises. The micelles attract each other resulting in the formation of the cubic phase. The second major effect accounts to the change of the interfacial energy. If an additive decreases the interfacial tension of the polymer-water interface, the formation of the micelles is thermodynamically less favorable. Thus, the micelles and the cubic phase disintegrate. However, the mechanism of the destabilizing effect of ionic surfactants was detected to be different. The surfactants form mixed micelles with the polymer introducing charge to the system. The ionic surfactants bind to the PPO-part and reduce the polymer´s amphiphilicity. Thereby, they disintegrate the micelle by forming small charged associates. In addition, the charged associates show enhanced electrostatic repulsion which hinders aggregation and thus the formation of the cubic phase according to the DLVO-theory (**Fig. 6**). It was also found that these described effects of additives on the cubic phase can overlay each other. This led to an optimal concentration for stabilizing the cubic phase in the case of 1,2-propylene glycol, glucose, and maltose.

Acknowledgements

We sincerely thank professor Dr. M. Lindemann and professor Dr. A. Wanninger from the University of Applied Sciences Niederrhein for mentoring the Bachelor thesis and the colleagues of the research departement Physical Chemistry at Henkel AG & Co. KGaA for their support.

Literature

[1] *T. Hellweg,* "Block Copolymer Surfactant Mixtures in Aqueous Solution: Can we Achieve Size and Shape Control by Co-Micellization?," in Self Organized Nanostructures of Amphiphilic Block Copolymers II, A. H. E. Müller and O. Borisov, Eds. Springer Berlin Heidelberg, 2010, pp. 1–27.

[2] *K. Kaizu* and *P. Alexandridis*, "Micellization of polyoxyethylene-polyoxypropylene block copolymers in aqueous polyol solutions," J. Mol. Liq., vol. 210, pp. 20–28, 2015, doi: 10.1016/j.molliq.2015.04.039.

[3] *P. R. Desai, N. J. Jain, R. K. Sharma,* and *P. Bahadur,* "Effect of additives on the micellization of PEO/PPO/PEO block copolymer F127 in aqueous solution," Colloids Surfaces A Physicochem. Eng. Asp., vol. 178, no. 1–3, pp. 57–69, 2001, doi: 10.1016/S0927-7757(00)00493-3.

[4] *P. Alexandridis* and *T. A. Hatton,* "DS thermodynamics , structure , dynamics , and modeling," Colloids Surfaces A Phisicochemistry Eng., vol. 7757, 1995.

[5] *A. Pitto-Barry* and *N. P. E. Barry*, "Pluronic® block-copolymers in medicine: From chemical and biological versatility to rationalisation and clinical advances," Polym. Chem., vol. 5, no. 10, pp. 3291–3297, 2014, doi: 10.1039/c4py00039k.

[6] *E. Hecht* and *H. Hoffmann*, "Interaction of ABA block copolymers with ionic surfactants in aqueous solution," Langmuir, vol. 10, no. 1, pp. 86–91, Jan. 1994, doi: 10.1021/la00013a013.

[7] *R. Ivanova, P. Alexandridis,* and *B. Lindman*, "Interaction of poloxamer block copolymers with cosolvents and surfactants," Colloids Surfaces A Physicochem. Eng. Asp., vol. 183–185, pp. 41–53, 2001, doi: 10.1016/S0927-7757(01)00538-6.

[8] *J. Jansson, K. Schillén, G. Olofsson, R. Cardoso da Silva,* and *W. Loh*, "The Interaction between PEO-PPO-PEO Triblock Copolymers and Ionic Surfactants in Aqueous Solution Studied Using Light Scattering and Calorimetry," J. Phys. Chem. B, vol. 108, no. 1, pp. 82–92, 2004, doi: 10.1021/jp030792u.

[9] *X. Mi et al.*, "Introduction of Anionic Surfactants to Copolymer Micelles: A Key to Improving Utilization Efficiency of P123 in Synthesis of Mesoporous Aluminosilicates," Ind. Eng. Chem. Res., vol. 56, no. 25, pp. 7224–7228, 2017, doi: 10.1021/acs.iecr.7b01142.

[10] *G. K. S. Prameela et al.,* "Physicochemical perspectives (aggregation, structure and dynamics) of interaction between pluronic (L31) and surfactant (SDS)," Phys. Chem. Chem. Phys., vol. 17, no. 45, pp. 30560–30569, 2015, doi: 10.1039/c5cp04910e.

[11] *R. Sharma* and *P. Bahadur,* "Effect of Different Additives on the Cloud Point of a Polyethylene Oxide-Polypropylene Oxide-Polyethylene Oxide Block Copolymer in Aqueous Solution," J. Surfactants Deterg., vol. 5, no. 3, pp. 263–268, 2002, doi: 10.1007-S11743-002-0226-9.

[12] *R. C. Da Silva* and *W. Loh,* "Effect of additives on the cloud points of aqueous solutions of ethylene oxide-propylene oxide-ethylene oxide block copolymers," J. Colloid Interface Sci., vol. 202, no. 2, pp. 385–390, 1998, doi: 10.1006/jcis.1998.5456.

[13] *S. Förster, V. Abetz,* and *A. H. E. Müller,* "Polyelectrolyte Block Copolymer Micelles," 2012, pp. 173–210.

[14] *R. Ganguly* and *V. K. Aswal*, "Improved micellar hydration and gelation characteristics of PEO-PPO-PEO triblock copolymer solutions in the presence of LiCl," J. Phys. Chem. B, vol. 112, no. 26, pp. 7726–7731, 2008, doi: 10.1021/jp801940m.

[15] *T. Liu* and *B. Chu*, "Formation of homogeneous gel-like phases by mixed triblock copolymer micelles in aqueous solution: FCC to BCC phase transition," J. Appl. Crystallogr., vol. 33, no. 3 I, pp. 727–730, 2000, doi: 10.1107/S0021889899013369.

[16] *H. Takahashi, A. Matsuo,* and *I. Hatta*, "Effects of salt on the lamellar and bicontinuous cubic phases of fully hydrated monoacylglycerol (monoelaidin)," Phys. Chem. Chem. Phys., vol. 4, no. 11, pp. 2365–2370, 2002, doi: 10.1039/b110852b.

[17] *Y. L. Wu, R. Sprik, W. C. K. Poon,* and *E. Eiser,* "Effect of salt on the phase behaviour of F68 triblock PEO/PPO/PEO copolymer," J. Phys. Condens. Matter, vol. 18, no. 19, pp. 4461–4470, 2006, doi: 10.1088/0953-8984/18/19/002.

[18] *N. J. Jain, V. K. Aswal, P. S. Goyal,* and *P. Bahadur,* "Micellar Structure of an Ethylene Oxide–Propylene Oxide Block Copolymer: A Small-Angle Neutron Scattering Study," J. Phys. Chem. B, vol. 102, no. 43, pp. 8452–8458, Oct. 1998, doi: 10.1021/jp982568o.

[19] *M. Ben Henda* and *A. Gharbi,* "Temperature, concentration and salt effect on F68 tri-block copolymer in aqueous solution: Rheological study," Polym. Sci. - Ser. A, vol. 59, no. 3, pp. 445–450, 2017, doi: 10.1134/S0965545X17030014.

[20] *Y. H. Hsu, H. W. Tsui, C. F. Lee, S. H. Chen,* and *L. J. Chen*, "Effect of alcohols on the heat of micellization of Pluronic F88 aqueous solutions," Colloid Polym. Sci., vol. 293, no. 12, pp. 3403–3415, 2015, doi: 10.1007/s00396-015-3662-0.

[21] *V. Patel et al.*, "Solubilization of hydrophobic alcohols in aqueous Pluronic solutions: Investigating the role of dehydration of the micellar core in tuning the restructuring and growth of Pluronic micelles," Soft Matter, vol. 9, no. 31, pp. 7583–7591, 2013, doi: 10.1039/c3sm50600b.

[22] *Y. Ding, Y. Wang,* and *R. Guo*, "Diffusion Coefficients and Structure Properties in the Pluronic F127/ n -C 4 H 9 OH/H 2 O System," J. Dispers. Sci. Technol., vol. 24, no. 5, pp. 673–681, Jan. 2003, doi: 10.1081/DIS-120023812.

[23] *P. Parekh et al.*, "Butanol solubilization in aqueous F127 solution: Investigating the enhanced micellar solvation and consequent improvement in gelation characteristics," Colloids Surfaces B Biointerfaces, vol. 114, pp. 386–391, 2014, doi: 10.1016/j.colsurfb.2013.10.030.

[24] *B. Sarkar, J. Lakshmichand,* and *P. Alexandridis*, "Self-assembly of amphiphilic block copolymers in ternary solvent mixtures: Lyotropic liquid crystalline phase behavior and structure," Macromol. Chem. Phys., vol. 213, no. 23, pp. 2514–2528, 2012, doi: 10.1002/macp.201200438.

authors

B.Sc. Janine Birnbach[a,b]
M.Sc. Jorge Esteban[c]
Prof. Dr. Thomas Hellweg[c]
Dr. Peter Schmiedel[b]

[a]Faculty of Chemistry, University of Applied Sciences Hochschule Niederrhein
Adlerstraße 28-32, 47798 Krefeld, Germany

[b]Henkel AG & Co. KGaA
Henkelstraße 67, 40589 Düsseldorf, Germany

[c]Physical and Biophysical Chemistry, Department of Chemistry, Bielefeld University
Universitätstraße 25, Bielefeld, Germany

IKW

The ecological baggage of a white cotton T-shirt in Germany

What contributes to its eco assessment?

The study „The lifecycle of a T-shirt – an eco assessment" by the Technical University Berlin describes the possible environmental impacts of the lifecycle of a white T-shirt – ranging from cotton cultivation and T-shirt manufacture to 44 x washing & drying and disposal. This study was mandated by the IKW (German cosmetic, toiletry, perfumery and detergent association) and high-lights this: Cotton production and T-shirt manufacture decisively determine most of the examined possible effects on the environment, e.g. land use, freshwater use, abiotic resource depletion potential as well as negative effects on waters and their organisms. The manufacture of the T-shirt and its use phase – i.e. washing & drying of the T-shirt – have a relevant influence on the global warming potential and the water depletion potential. In order to significantly reduce the environmental impacts of washing & drying in private households, the following applies: wash at low temperatures, precisely dose the detergent, load the washing machine to full capacity, and dry laundry outdoors.

On behalf of the IKW, the Technical University Berlin carried out the study "The lifecycle of a T-shirt – an eco assessment" which sums up the most important environmental impacts of a garment's lifecycle. The assumptions made for this study were as realistic as possible. For example, a standard commercial cotton T-shirt (white) was examined which was manufactured outside Europe, bought in Germany, worn, washed & dried 44 x, and finally disposed. Such a cotton T-shirt weighs about 150 g. The manufacture of the T-Shirt, the distribution and the textile care during its use phase as well as the disposal require quantities of resources (e.g. water) that exceed its weight many times over. Furthermore, substances are released during its lifecycle, e.g. greenhouse gases which correspond to 3.7 kg of carbon dioxide (CO_2). Here, it was assumed that consumers do not load their washing machines to capacity but only with 3.5 kg of laundry, dose 55 ml of liquid detergent per wash, and dry only every 10th load in a tumble dryer – the rest in fresh air.

Environmental impacts

Ecological assessments use the generic term "environmental impacts" to describe the effects of substances or processes on certain fields of the environment. This includes possible direct and indirect effects, which are caused, for example, by the manufacture of the T-shirt, washing machine or detergent. This study examined, inter alia, the following environmental impacts:

- Possible effects of the T-shirt's manufacture, use and disposal on global warming are referred to as global warming potential.
- The consumption of raw materials such as ores, metals or minerals, which are needed for the manufacture of the T-shirt, washing machine, detergent and tumble dryer and for the providing of energy and water, are summarised under the abiotic resource depletion potential.
- The possible impacts on the quality and function of soils (e.g. through changes in use or sealing and for the extraction and processing of raw materials) are described by the impact category land use.
- The acidification potential describes the impact of acidifying substances, which are released in the T-shirt's manufacture or textile care, on soils and waters.
- The measuring approach water depletion potential is the environmental impact of the consumption of water as a resource. Here, the use of freshwater (e.g. for cotton cultivation or washing during the use phase) is taken into account. However, the quantities of used freshwater that are returned into waters (e.g. after use in power plants as cooling water or to drive turbines or after purification in sewage treatment plants) are also included in the considerations.
- The environmental impact fresh-water aquatic ecotoxicity potential is the term to describe the negative impacts that releases of substances can have on waters and their organisms.

In ecological assessments, it is not permitted to weigh different environmental impacts against each other.

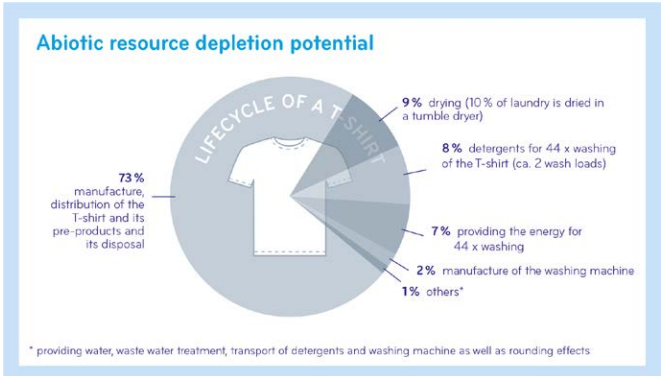
When a T-shirt is newly purchased, it already has the "ecological baggage" of environmental impacts due to its manufacture and distribution. When it is subsequently used – and thus also washed & dried – this further adds to the T-shirt's "ecological baggage". The most important results of the eco assessment are described in the following 10 key messages:

1 The environmental impacts of a T-shirt's lifecycle are mainly determined by cotton cultivation and T-shirt manufacture.

Referred to the entire lifecycle of a T-shirt which, hypothetically, is washed & dried only once, the manufacture and distribution of the T-shirt and its pre-products as well as its disposal contribute 96% to the global warming potential, 98% to the water depletion potential, and 99% to the abiotic resource depletion potential. 1 x washing & drying has a share of only 4.2% and 1%, respectively, in these three environmental impacts.

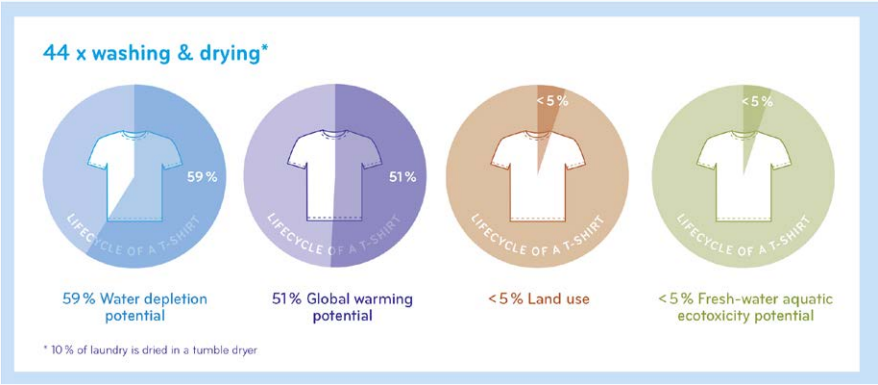
3 The consumption of raw materials such as ores, metals or minerals in the T-shirt's lifecycle is largely attributable to its manufacture.

Around 73% of the abiotic resource depletion potential respectively raw materials such as metals, ores and minerals contributes to the manufacture and distribution of the T-shirt and its pre-products and for its disposal; drying of the T-shirt accounts for 9%, assuming that it is mostly dried in fresh air and only every 10th time in a tumble dryer. In 44 x washing of the T-shirt, a maximum of 8% in the consumption of these raw materials falls to the share of detergent manufacture, Further 7% are required for providing the energy for the washing machine; the manufacture of the washing machine accounts for just under 2%.



2 44 x washing & drying of a T-shirt contributes roughly as much to the global warming potential as the T-shirt's manufacture, distribution and disposal.

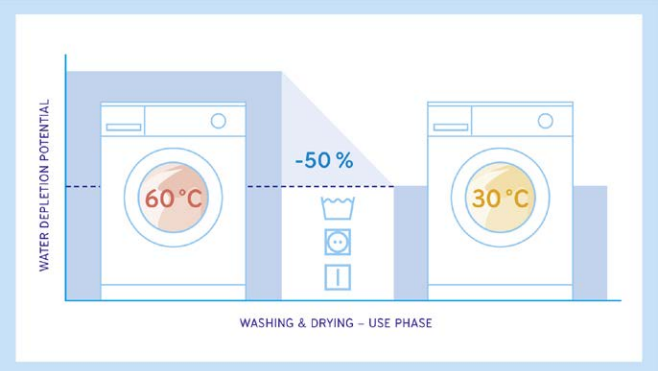
Well over half (51%) of the global warming potential is attributable to 44 x washing & drying of the T-shirt during its average lifespan or duration of wearing, assuming that only every 10th time a tumble dryer is used. 49% of the global warming potential is determined by cotton production and the T-shirt's manufacture, distribution and disposal. 44 x washing & drying during the entire lifecycle contributes 59% to the water depletion potential. 44 x washing & drying contributes less than 5% each to the environmental categories land use and fresh-water aquatic ecotoxicity potential while the T-shirt's manufacture, distribution and disposal account for over 95%.



4 Consumers can decisively reduce the environmental impacts during the use phase of the T-shirt by **washing at low temperature.**

Reducing the washing temperature from 60°C to 30°C leads to less energy consumption. This lowers the global warming potential during the use phase by ca. 37%.

In fact, the amount of water used by a washing machine in a particular wash programme (e.g. "cotton") does not depend on the temperature. All the same, washing at lower temperature reduces the use of water. This is because water is also needed to provide electricity, e.g. to drive turbines and for the cooling of power plants. Consequently, the contribution of the use phase to water depletion potential is cut almost by half if laundry is washed at 30°C instead of 60°C.



5 By **loading the washing machine** as full as possible, consumers can decisively reduce the T-shirt's environmental impacts during its use phase.

If a washing machine, in which up to 7 kg of laundry can be washed, is used at full load and not at half load, this can reduce the global warming potential during the use phase by 45%.

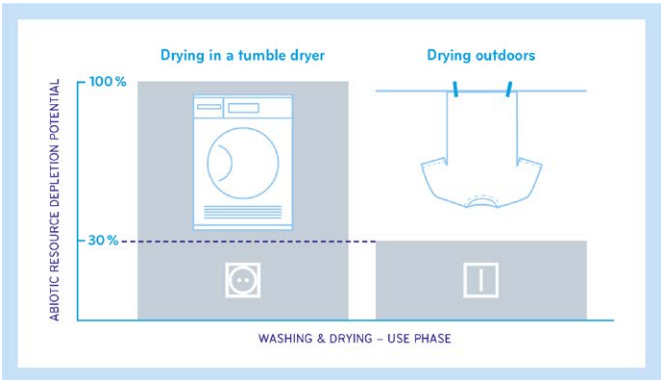
The water depletion potential, too, is roughly cut by half during the use phase if the washing machine is loaded to full capacity.

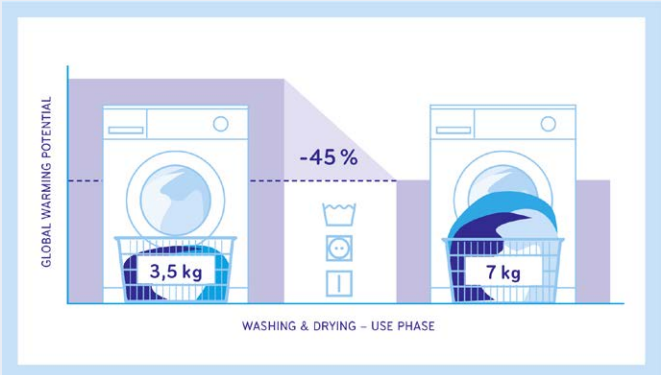
6 During the T-shirt's use phase, **detergent dosing** influences, in particular, the abiotic resource depletion potential.

If almost twice the amount of detergent (100 ml instead of 55 ml) is used per wash cycle, the abiotic resource depletion potential rises by 22% during the use phase. At the same time, the following environmental impacts increase: acidification potential by 19% and global warming potential by 6%.

7 **Drying outdoors** significantly reduces the environmental impacts during the T-shirt's use phase.

Consumers can greatly influence the environmental impacts in the drying of the T-shirt. The manufacture of the tumble dryer and providing energy and water contributes directly and indirectly to the abiotic resource depletion potential. Referred to the use phase, abiotic resource depletion potential in private households, where the T-shirt is washed 44 x and dried every time exclusively in a tumble dryer, is thus around three times higher than in private households where the T-shirt is washed 44 x but dried exclusively outdoors. The global warming potential and the water depletion potential are more than twice as high.

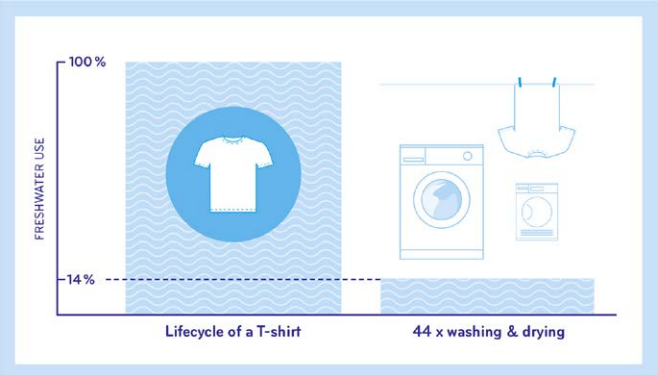




8

Throughout the T-shirt's lifecycle, freshwater use is dominated by the manufacture of pre-products for the T-shirt.
44 x washing & drying of the T-shirt has a share of 13% in freshwater use.

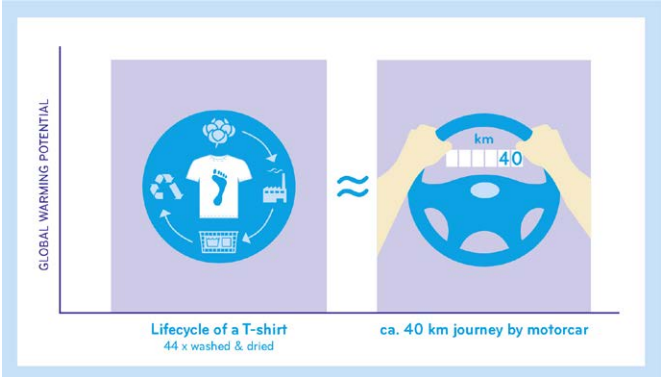
In total, 1,670 litres of freshwater are used for the entire lifecycle of a T-shirt that is washed & dried 44 x. Out of this total, the manufacture of the T-shirt's pre-products (e.g. cotton) requires 1,370 litres, which accounts for 82% of freshwater use throughout the entire lifecycle. 55 litres of freshwater (3%) are used for the actual manufacture of the T-shirt. 221 litres (13%) of freshwater are needed for the washing process. 17 litres (1%) are necessary for drying, assuming that only every 10th time the tumble dryer is used. As regards freshwater use, shares of freshwater also go into the providing of electrical energy for the washing process and drying in a tumble dryer, e.g. for cooling in power plants and driving turbines.



10

A calculation independent of the TU Berlin study shows that a journey of almost 40 km in an average motorcar has the same global warming potential as the entire lifecycle of a T-shirt.

The global warming potential of a T-shirt – from manufacture to 44 x washing & drying and disposal – is equivalent to 3.7 kg of carbon dioxide (CO_2). A 40 km journey in an average, petrol-driven motorcar has roughly the same global warming potential. This comparison was carried out independently of the TU Berlin study; it only serves to illustrate and categorise the result for global warming potential. The starting point for this comparison is an assumed carbon dioxide emission of an average motorcar of 95 g of carbon dioxide [1] per kilometre driven.

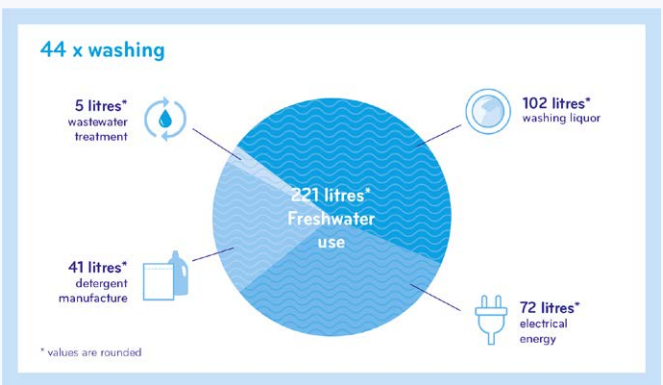


[1] Regulations (EC) No 443/2009 and (EC) No 510/2011 to reduce CO2 emissions of passenger cars and light-duty vehicles – applicable in the European Union – lay down that from 2020 in the EU newly registered vehicles may emit on average only 95 g of CO_2/km.

9

For washing the T-shirt 44 x, a total of 221 litres of freshwater are used – out of this total, less than half (102 litres) for the washing liquor and just under a third (72 litres) for providing electrical energy.

To wash 44 x a T-shirt that weighs ca. 150 g, roughly as much water is needed for the washing liquor as for two wash loads with about 3.5 kg of laundry each, i.e. ca. 102 litres of freshwater. Add to this just under 72 litres of freshwater for providing electrical energy, e.g. for cooling in power plants and driving turbines. Around 41 litres of freshwater are used to manufacture the detergent for two wash loads (110 ml). Additionally, waste water treatment requires nearly 5 litres for two of these wash loads.



Tips for environmentally sound laundry care

1. The **right care contributes to preserving the T-shirt's value** so that it can ideally be washed and worn more than 44 x. For this purpose, the care instructions in labels should be observed and textiles should be washed & dried accordingly.

2. The T-shirt should be washed preferably at **low temperatures** to avoid unnecessary energy use. For the hygiene of the washing machine, a bleach-containing heavy duty detergent in solid form (powder, granule, tab) should be used for washing at 60°C at least once a month. Only solid heavy duty detergents contain bleaching agents.

3. If the **washing machine** is **loaded as full as possible,** it works particularly economically. "Loaded as full as possible" means the following for a washing machine with a 7 kg loading capacity:
   - With a cotton programme – 7 kg of dry laundry.
   - With an easy-care programme – a maximum of 4 kg of dry laundry.
   - With a wool programme – a maximum of 1.5 kg of dry laundry.

   If different items of laundry are combined with each other, they should be washed with the programme and detergent type for the most sensitive laundry item.

4. Follow the **dosing instructions on detergent packs.** The amount of detergent needed depends on the degree of soiling, load of the washing machine and water hardness.
   - Avoid over- and underdosing of the detergent.
   - Choose the right detergent for each type of laundry (heavy duty detergent, colour or easy-care or wool detergent).
   - Information about the water hardness range can be obtained from water suppliers or landlords.

5. **Laundry should preferably be dried outdoors.** If this is not possible due to weather conditions, drying in an unheated, well-ventilated room is recommended. Good ventilation is important to avoid mould formation.

What is behind it?

An eco-balance is also called a lifecycle assessment (LCA). An eco-balance describes the environmental impacts of a product, process or service across its entire lifecycle. The lifecycle of a product can be characterised, for example, by individual stages or lifecycle phases: manufacture and distribution of the pre-products (e.g. cotton, yarns), manufacture and distribution of the main product (e.g. T-shirt), use phase of the main product (e.g. washing & drying the T-shirt), disposal of the main product (e.g. thermal, material or mechanical recycling of the T-shirt and accompanying substances).
A lifecycle analysis can be used to identify product-related environmental impacts, assign them to the lifecycle phases and derive recommendations for action. In this study, the environmental impacts at each stage of the lifecycle refer to a white cotton T-shirt which is washed 44 x, each time in a washing machine with a total load of 3.5 kg, with 55 ml of an average liquid detergent and at an average washing temperature of 43.3°C. Washing the T-shirt 44 x corresponds to roughly two washing machine loads. Drying was assumed to be done in a tumble dryer in only 10% of cases; in all other cases, drying was outdoors.

The white cotton T-shirt is the so-called functional unit for the eco-balance to which the environmental impact calculations refer.

Environmental impacts describe the effects of a process (e.g. manufacture of a T-shirt) on the environment. The eco-balance of a standard commercial cotton T-shirt (white) included, inter alia, the examination of the following environmental impacts:

- **Global warming potential:** The relative contribution of a substance or process to the greenhouse gas effect.
- **Abiotic resource depletion potential such as metals, ores, minerals, stones, gravel etc:** Use of non-fossil and non-renewable resources ("elementary resource consumption").
- **Land use:** For example, in consequence of agricultural activities or caused by the utilisation and sealing of soil in the production and processing of raw materials (e.g. in order to obtain raw materials, plants are cultivated on agricultural areas or mineral oil is produced).
- **Acidification potential:** This is due to the emissions of acid-forming substances. Both terrestrial and aquatic ecosystems can be damaged by acidification.
- **Danger of over-fertilisation ("terrestrial eutrophication potential"):** Release of nutrients that can lead to an over-fertilisation of soils (terrestrial ecosystems) and adjacent waters (aquatic ecosystems).
- **Water depletion potential ("Potential water scarcity"):** This takes into account, inter alia, the use of freshwater for the respective processes in the lifecycle but also the proportionate backflows of used freshwater quantities in water catchment areas, e.g. after passing through power plants (cooling water, driving turbines) or after successful purification in waste water treatment plants. Thus, the environmental impact Water depletion potential is to measure water scarcity.

Picture Credits shutterstock.com: S. 45 hvoya, S. 47 valkoinen, S. 49 kazoka

- **Fresh-water aquatic ecotoxicity potential:** Entry or release of substances that can have a harmful effect on freshwater organisms.

The environmental impacts of the T-shirt were appraised on the basis of a lifecycle model which was developed especially for this purpose and depicts the entire lifecycle of a white cotton T-shirt used in Germany, i.e. ranging from the use of material or energy resources for the T-shirt's manufacture along global production routes to its use (textile care), recycling and disposal in Germany. The specific environmental impacts of the T-shirt were determined using the eco-balance software GaBi 8.7, 2018. This software system includes pre-set standard parameters e.g. for upstream and downstream process chains (such as cotton production, fibre production, transport) of raw material and input production, taking into account the geographical reference. Further parameters were obtained in literature searches and expert discussions and incorporated in the model.

The eco assessment (lifecycle assessment) of a standard commercial cotton T-shirt (white) was completed in 2019 and followed the international LCA standards ISO 14040 and ISO 14044.

IKW"
HAUSHALTSPFLEGE"
KOMPETENZPARTNER IM IKW

Home Care Department
The German Cosmetic, Toiletry, Perfumery and Detergent Association (IKW)
Mainzer Landstraße 55, 60329 Frankfurt am Main, Germany
www.haushaltspflege.org

The complete study report of TU Berlin in German language ("Ökobilanzielle Bewertung des Lebensweges eines handelsüblichen weißen Baumwolle T-Shirts in Deutschland") can be accessed at this web address:
https://www.ikw.org/ikw-english/home-care-topics/detail/the-lifecycle-of-a-t-shirt-an-eco-assessment-670/


Stefan Fellner

ALPIN HEILMOOR EXTRACT
Power of Nature Reinterpreted

Interview with Stefan Fellner, Managing Director and Founder, PREMIUM ORGANIC

Mr. Fellner, you and your company PREMIUM ORGANIC recently launched ALPIN HEILMOOR EXTRACT (AHE), a new active ingredient for skin care.

What is AHE?

ALPIN HEILMOOR EXTRACT™ – AHE for short – is Heilmoor in its purest, finest and most concentrated form as a dark, micronized powder.

I grew up in the south of Austria – at 500m above sea level and surrounded by moor. This was always there for me as a matter of course and I never thought that it would become my center of life. But at some point, I simply became curious. I wanted to know what this force of nature really had to offer right before my eyes. After all, Heilmoor has been used for hundreds of years, primarily as a pack or bath – mainly for illnesses and pain in the musculoskeletal system.

So, my company PREMIUM ORGANIC, started to take a closer look at this European natural medicine and tradition. In cooperation with partners from all over Europe we started our research. In the course of many years of development, we not only learned a lot of exciting things about the substance Heilmoor, but also developed AHE. In this active ingredient we now bundle the most potent parts of the Heilmoor and make them available in a new form: concentrated and as a powder. Unlike classic Heilmoor, AHE can be used not only for its thermo-physical properties (in the form of baths and packs), but also in the field of dermocosmetics to show what it can do. 100% nature – but interpreted by us in a completely new way.

ALPIN HEILMOOR EXTRACT™ is a patented, multifunctional active ingredient for cosmetic and personal care products, created thanks to a completely new process and produced by our partners in Germany and Austria.

What are the advantages of this active ingredient?

The great innovation of ALPIN HEILMOOR EXTRACT™ is definitely its brand-new form: micronized powder instead of wet peloid. This is also where the biggest advantages of AHE lie.

The goal of PREMIUM ORGANIC is to develop and produce active ingredients that interpret nature in a modern and scientifically sound way. With AHE, we have thus succeeded in producing our first active ingredient, which is not only proven to be effective in a variety of ways, but also opens up completely new areas of application and new paths.

Compared to its origin, AHE is pure and reduced to the most potent components – free of water and any microbiological contamination. Thanks to our innovative and patented process, we are able to provide the natural substance Heilmoor in a consistent form and quality: as a powder. From the very beginning, we have taken both scientific and ecological standards into account and thought them through. In extensive studies, we have also been able to scientifically prove the effectiveness of Heilmoor for the first time. AHE thus offers completely new areas of application for Heilmoor, both for leave-on and rinse-off products in the field of dermocosmetics and personal care.

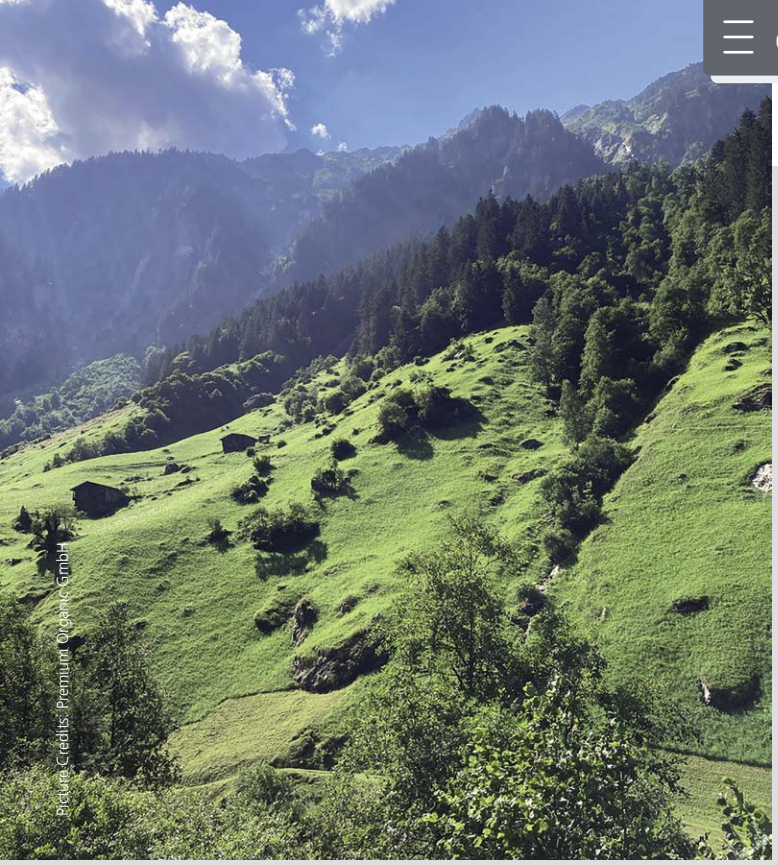
AHE is also 100% natural as well as COSMOS-, ECOCERT-, NATRUE- and VEGAN-certified. At our company PREMIUM ORGANIC, we attach great importance to sustainability at all stages of the process, from extraction to production of the active ingredient.

What efficacy do you praise for AHE?

Heilmoor – as already mentioned – has been used in Europe for centuries for health purposes. However, due to its good heat retention properties, the focus was mainly on complaints in the area of joints, muscles or rheumatic diseases and sports injuries. With AHE, we wanted to show – and above all prove - what additional efficacies Heilmoor has to offer. In *in-vitro* and *in-vivo* studies, we were able to prove that ALPIN HEILMOOR EXTRACT™ has multiple benefits for sensitive and problem skin, as well as in the area of inflamm-aging.

The skin is a sensitive system whose balance is always in danger due to external factors but also stress and aging processes. AHE helps here in many ways and has a positive effect on several important parameters that make up a healthy, pure and above all even mature beautiful skin.

Not only does AHE maintain the skin microbiome – which has been recognized in recent years as an essential factor in healthy skin – but there is evidence that AHE helps and supports to restore its balance. Our proven Cleansing & Anti-Pollution effects show that AHE can rid the skin of stress and environmental factors. The skin barrier – our external protection, so to speak – is improved and its integrity strengthened by AHE. We have also demonstrated anti-inflammatory and soothing efficacies. Significant wound healing and anti-acne efficacy show that ALPIN HEILMOOR EXTRACT™ can also be used on problem skin.



Picture Credits: Premium Organic GmbH

The antioxidant and pro-aging effects are also impressive. For example, the particularly positive effect on the sensitive area around the eyes – which not only looks more radiant, but also less puffy – should be emphasized. Further positive effects on mature and sensitive skin concerning elasticity and moisture complete this picture. Inflamm-aging was also proven by the suppression of various inflammatory markers.

How were these claims confirmed in the test?
From the very beginning, it was important to our company, PREMIUM ORGANIC, not only to open Heilmoor to new areas of application, but also to place its effectiveness on a scientifically sound basis. In doing so, we rely exclusively on external laboratories in the EU. A series of *in-vitro* tests, for example, showed the positive properties in the area of wound healing, membrane barrier or skin inflammation. In vivo efficacy studies again confirmed the cleansing and soothing effect of AHE, as well as a convincing anti-acne efficacy. It is also remarkable how well AHE was tolerated and appreciated by the test persons. The results of our studies have already been widely published. Since some of the results surprised even us positively, new studies are already underway.

An important criterion in cosmetics is sustainability. What is AHE derived from? And what is the manufacturing process like?
Sustainability has played a major role in our company from the very beginning. Through transparency and in close contact with experts, we work to make the entire process from development to production of our active ingredients long-term and sustainable.

AHE is extracted from an accredited Heilmoor deposit in the south of Austria. At this point it should be mentioned that only about 10% of all Heilmoor deposits are at all suitable for the extraction of AHE due to their composition (proportion of humic acids, etc.). Environmental experts were involved in the extraction and processing right from the start. In this way, we have developed a process in which renaturation plays a major role. In the course of gentle and sustainable quarrying, a natural refuge for flora and fauna is created through biotopes and backfilling. PREMIUM ORGANIC attaches great importance to transparency at all stages of the process and supports this with certifications. AHE is COSMOS, ECOCERT, NATRUE and VEGAN certified. We are currently working on making our company completely CO_2 neutral.

For which product formulations is AHE suitable?
One of the biggest advantages of ALPIN HEILMOOR EXTRACT™ is that the active ingredient is so easy to use, yet so versatile. Both leave-on and rinse-off products can be made with it. In an initial series of frame formulations, we have designed, for example, cleansing, peel-off and purifying masks, day creams, BB creams, cleansing gels, and also hair shampoos and conditioners. Depending on the concentration used, AHE colors the products light to dark gray. However, none of this is visible on the skin.
AHE can also be combined very well with other ingredients and is very unproblematic to use and easy to formulate. For example, no pre-dispersion is necessary. Anti-aging, detox or skin care products: Due to its good skin compatibility – even with sensitive or problem skin – and its positive properties, AHE is suitable for a wide range of applications.
Products in hair and scalp care as well as especially for men are currently being developed by customers of ours. We ourselves are always amazed at how versatile ALPIN HEILMOOR EXTRACT™ can be used and are pleased that the active ingredient obviously encourages experimentation and testing. AHE not only convinces with its efficacy and good tolerability, it is also in line with the spirit of the times and is scientifically state-of-the-art. Moreover, AHE is appreciated and very well received by formulators and customers alike. This shows us that we have achieved our goal of developing an uncomplicated and versatile natural active ingredient.

www.premium-organic.com

Picture Credits: WALA Heilmittel GmbH

Hydrolates: Finest Floral Waters with Gentle Fragrances

Sophia Steinmetz

Up to now, hydrolates – also called flower waters – have been regarded as by-products that are created during the production of essential oils. We have a different point of view. We realise and appreciate the importance of gentle fragrances as a premium resource for natural cosmetics, cleansing products, and aromatherapy. We, therefore, draw on the many years of expertise of our employees and produce water-based hydrolates explicitly – not as a by-product – with finest essential oils.

We place particular emphasis on certified process quality (ISO 9001) and fully traceable supply chains of essential oils from organic origin. Against the backdrop of the "Fridays for Future" movement and a growing number of critical consumers, end-to-end sustainable environmental awareness and supply chain transparency have become the "state of the art", especially in the raw materials sector. "Eco" is on everyone's lips. Certified environmental awareness, for example according to ISO 14001, is therefore becoming a predicate. The purchase of 100 percent local green electricity emphasises one's own climate awareness on top of that. This environmental approach is one reason why naturamus has decided to produce hydrolates with essential oils.

Since we are in charge of the production management and do not import tons of fresh plants by ship, we significantly reduce our CO_2eq emissions. Instead, we produce hydrolates in-house. This approach makes us independent of partly unstable supply chains as well as seasonal availability and ensures local jobs in the Swabian SME sector.

Holistic sustainability also means that resources are preserved and products are used in their entirety. Long-term storage including destruction of hydrolates is, therefore, not an option for naturamus. Instead, we produce hydrolates on demand according to the customer's wishes throughout the year – regardless of the harvest time of fresh plants. This allows us to minimize storage times and to exactly meet the demand with our production.

We achieve this by using essential oils instead of herbs and flowers. The quality of these products depends on external factors such as climate and weather conditions. On top of that, producers of genuine hydrolates are confronted with the challenge of microbial contamination. During the time-honoured process of steam distillation to extract essential oils from plant and herbal extracts, tiny particles are often carried along with the steam flow. As a result, they can pass into the distillate. These particles provide a breeding ground for microorganisms, making the finished products more susceptible to microbial contamination. This problem can be countered preventively with the help of essential oil processing. Adherence to strict hygiene standards and close-meshed quality control – including microbiological laboratory tests per batch – support this quality standard. The use of preservatives and ethanol is, thus, unnecessary.

naturamus offers a wide-ranging portfolio of conventional as well as organic, (analogue)-certified hydrolates from its own production. We also expand our portfolio upon customer request. Salary processing is also possible.

www.naturamus.de

Melissa officinalis

Rosa damascena

Orange blossom

COSMETIC INGREDIENTS & FORMULATIONS GUIDE 2019

The speed of innovations in the cosmetics industry continues to be fast-paced.

Who has new ideas and formulations? Which marketing strategy do I pursue with my product? What is the consumer trend? Which topics are relevant?

Many new products and technologies were presented at the various trade fairs during the past year. The Cosmetic Ingredients and Formulations Guide 2019 presents some of these new products and cosmetic topics with a large number of formulation examples and provides an overview of the suppliers of active substances and ingredients.

Find the latest inspirations and trends in the Cosmetic Ingredients and Formulations Guide 2019.

19th Edition • Color Illustrations • 98 Formulations • 248 Pages

Order Form

	Order-No.	*ISBN*
☑ ____ Copy(ies) **Cosmetic Ingredients & Formulations Guide 2019**	V50010694	978-3-87846-306-1
Price: EUR 75,- (+ Shipping)		

Company _____ Customer ID _____

Name _____ Tel. _____

Street/P.O. Box _____ Fax _____

Zip Code, City _____ E-Mail _____

VAT Id. Nr. (if EU) _____ Date, Sign _____

☐ PayPal: vci@sofw.com

☐ Invoice/Bank Transfer

☐ Credit card ☐ Visa ☐ Amex ☐ Mastercard

Card-No. _____ Card Code _____

Exp. Date _____

Cardholder´s Name _____

SOFW BOOKSHOP

Verlag für chemische Industrie H. Ziolkowsky GmbH
Dorfstr. 40
86470 Thannhausen, Germany
Tel.: +49 8281 79940-20
Fax: +49 8281 79940-50
✉ bookshop@sofw.com
www.shopsofw.com

Ageless Firming Body Lotion

SC-FR-20-BC-50892-03

BASF We create chemistry | Care Creations

Phase	Ingredients	INCI	% by weight	Function
A	Water, demin.	Aqua	79.17	
	Glycerin	Glycerin	2.00	Humectant
	Euxyl PE 9010 (Schülke)	Phenoxyethanol, Ethylhexylglycerin	1.00	Preservative
B	Rheocare® C Plus	Carbomer	0.20	Rheology modifier
C	Eumulgin® VL 75	Lauryl Glucoside, Polyglyceryl-2 Dipolyhydroxystearate, Glycerin	2.00	Emulsifier (O/W)
	Lanette® O	Cetearyl Alcohol	1.00	Consistency agent
	Myritol® 331	Cocoglycerides	3.00	Emollient
	Cetiol® 868	Ethylhexyl Stearate	3.00	Emollient
	Cetiol® CC	Dicaprylyl Carbonate	4.00	Emollient
	Hydrolite 8 (Symrise)	Caprylyl Glycol	0.50	Auxiliary
D	Cosmedia® Ace	Sodium Polyacrylate, Dicaprylyl Carbonate, Polyglyceryl-3 Caprate	1.00	Rheology modifier
E	PeptAlde™ 4.0 BC10129	Aqua, Hydrolyzed Rice Protein, Citric Acid	2.00	Active ingredient
	Perfume*	Parfum	0.60	Fragrance
	Sodium Hydroxide (18% solution)	Sodium Hydroxide	0.53	pH Adjustment

Specifications:
pH value (20°C) 5.55
Viscosity (Brookfield; DV-I+; spindle 5; 10 rpm; 20°C) 15 000 - 20 000

Performance:
Additional performance has not been evaluated

Manufacturing Process:
Hot Process
Introduce phase B in phase A while stirring.
Heat phases A+B and C at 75-80°C.
Add phase C in phase A+B with low stirring for 10 minutes.
Then, add phase D in phase A+B+C under gentle stirring.
Cool down at room temperature and add the ingredients of phase E one by one.
Finally homogenize during 10 minutes.

Stability Test:
Conform 3 months at 4°C, RT, 40°C, 45°C

Additional information:
Perfume*: Aqua d'Eau RS68717 (Technicoflor) - no allergens to declare

Healthy Shampoo & Shower Gel 2 in 1

HB-FR-20-BC-50891-02

Phase	Ingredients	INCI	% by weight	Function
A	Water, demin.	Aqua	35.30	
	Sulfopon® 1216 G	Sodium Coco-Sulfate	8.00	Surfactant
B	Dehyquart® Guar HP	Guar Hydroxypropyltrimonium Chloride	0.50	Conditioning agent
	Water, demin.	Aqua	35.45	
	Citric Acid (50% solution)	Citric Acid	0.10	Neutralizing agent
C	Plantacare® 818 UP	Coco-Glucoside	13.30	Surfactant
	Lamesoft® PO 65	Coco-Glucoside, Glyceryl Oleate	1.00	Conditioning agent
	Sodium Benzoate	Sodium Benzoate	0.50	Preservative
D	Citric Acid (50% solution)	Citric Acid	2.20	pH Adjustment
	Sodium Chloride	Sodium Chloride	0.65	Rheology modifier
E	PeptAlde™ 4.0 BC10129	Aqua, Hydrolyzed Rice Protein, Citric Acid	2.00	Active ingredient
F	Perfume*	Parfum	1.00	Fragrance

Specifications:
pH value (23°C) 4.85
Viscosity (Brookfield; RVT; spindle 4; 10 rpm; 23°C) ~ 8000

Performance:
Additional performance has not been evaluated

Manufacturing Process:
1. Dissolve Sulfopon® in warm water (approx. 40°C) while stirring.
2. Add Dehyquart® Guar HP to water while stirring. Add citric acid and stir for 10 minutes.
3. Premix phase C, add phases A and B and stir until homogeneous.
4. Adjust pH and viscosity by using phase D.
5. Add phases E and F while stirring.

Perfume*:
Perfume*: 1% Aqua d'Eau RS68717 (Technicoflor, FR)

Stability Tests:
Stable 3 months at 4°C, RT, 40°C, 45°C

SEPAWA® CONGRESS 2021 News: SEPAWA Stays Virtual

Tannhausen/Berlin, Germany, February 18, 2021. COVID-19 will continue to be a major problem for organisers in 2021. Various industry and trade shows have already been cancelled or postponed, as reported by the Association of the German Trade Fair Industry (AUMA). The new mutations of the virus give rise to further uncertainties for future developments. Travel restrictions and quarantine regulations further contribute to planning uncertainty.

Therefore, the board of SEPAWA® e.V. has decided to hold the SEPAWA® CONGRESS 2021 exclusively on a virtual basis.

"This decision has been very difficult for us. However, we want to give our exhibitors and participants planning security. We definitely want to avoid a last-minute cancellation like we had to do in 2020. This would mean unnecessary costs and time lost for everyone involved. The health of our visitors and participants is always paramount, and at the moment we cannot predict with certainty the development of the pandemic, the vaccination status, or any hygiene regulations and participant restrictions in the autumn. Therefore, we think that the virtual option is the only right one. We hope that the current decision is in the interest of all participating companies and delegates," *Dr. Hans Jürgen Scholz*, 1st Chairman

Meet your business from wherever you are!

The experience with a virtual event in 2020 was very positive. The platform was consistently rated positively by the participants. In the meantime, the system has been continuously improved by the manufacturer and provided with new functions. Above all, the virtual exhibition area has undergone decisive innovations.

Meet your business from wherever you are!

„We are convinced that the virtual SEPAWA® CONGRESS 2021 will be successful. By committing to a digital event early on, there is enough time to prepare thoroughly for participation," explains *Robert Fischer*, SEPAWA® press officer.

Take the opportunity to present your latest scientific research results at SEPAWA® CONGRESS VIRTUAL 2021.

www.sepwa-congress.com

LANXESS headquarters in Cologne, Germany. Picture Credits: LANXESS

LANXESS Signs Contract to Acquire Emerald Kalama Chemical

Cologne/Germany, February 14, 2021. Specialty chemicals company LANXESS is accelerating its growth course and signed a binding agreement to acquire 100 percent shares in Emerald Kalama Chemical on February 14, 2021. The US-based company is a globally leading manufacturer of specialty chemicals, especially for the consumer segment, and is majority-owned by affiliates of the US private equity firm American Securities LLC.

The enterprise value of Emerald Kalama Chemical amounts to USD 1.075 billion. After deducting debt-like items, the purchase price is around USD 1.04 billion (EUR 867 million*), which LANXESS will finance from existing liquidity. The transaction is expected to be completed in the second half of 2021. It is still subject to approval by the relevant authorities.

In 2020, Emerald Kalama Chemical achieved sales of around USD 425 million and EBITDA pre exceptionals of approximately USD 90 million. Within three years following the completion of the transaction, LANXESS expects an additional annual EBITDA contribution of around USD 30 million (EUR 25 million*) from synergy effects. The acquisition will already be earnings per share accretive in the first fiscal year after its completion.

"We are gaining further momentum on our growth course. The businesses of Emerald Kalama Chemical are an ideal fit for us. We will further strengthen our Consumer Protection segment and open up new application areas with strong margins, for example in the food industry and animal health sector. In addition, we will also enlarge our presence in our growth region of North America. All this will make us even more profitable and stable," said *Matthias Zachert*, Chairman of the Board of Management of LANXESS AG.

Emerald Kalama Chemical employs approximately 500 employees worldwide and runs production sites in Kalama, Washington (USA), Rotterdam (Netherlands) and Widnes (Great Britain). Emerald Kalama Chemical generates around

45 percent of its turnover in North America. "Emerald Kalama Chemical has a very efficient setup, bundling all its production activities at only three sites. That is why we expect to integrate the new business very quickly," said *Zachert*.

Targeted portfolio expansion in the Consumer Protection segment

Emerald Kalama Chemical generates about 75 percent of its turnover with specialties in the consumer segment. These include preservatives for food, household and cosmetic applications, flavors and fragrances as well as products for animal nutrition. The remaining 25 percent of sales come from the specialty chemicals business for industrial applications, including the plastics and adhesives industries.
With the acquisition, LANXESS is pursuing a targeted expansion of its portfolio: The company has a strong position in the global business with antimicrobial active ingredients and preservatives, including for consumer protection products and animal hygiene. Examples are disinfectants effective against the Coronavirus or the African Swine Fever.

*Based on exchange rate of EUR/USD = 1.20.

www.lanxess.com

Symrise Upgrades Online Platform for Aroma Molecules

Holzminden/Germany | February 24, 2021. Symrise has completely revamped its online platform for fragrance and flavor raw materials. The Ingredient Finder at symrise.com (www.symrise.com/scent-and-care/aroma-molecules/ingredient-finder/) provides all the key information about the company's aroma molecules in the fragrance, flavor and pharmaceutical categories. The database is intuitive to use: Filter functions help users quickly find the desired data. Another advantage comes from its clear design. Symbols allow users to identify particularly renewable or high impact raw fragrance materials at a glance.

Symrise is presenting its aroma molecules in a new guise. The company provides detailed information about its product portfolio and makes all important data about its fragrances, flavors and pharmaceutical raw materials available in its Ingredient Finder. The modernized online platform scores with its clear design, which Symrise is now also using for printed compendiums and data sheets. Thanks to the clear structure, users can easily find aroma molecules.
"Technological progress provides companies with great opportunities. This also applies to the presentation of our own raw materials on our website," says *Dr. Marcus Eh*, Director Global Marketing Aroma Molecules at Symrise. "With the Ingredient Finder, we offer all relevant information about our aroma molecules on a modern online platform and thereby make them more visible." For the first time, the Ingredient Finder provides information on raw materials for pharmaceutical products. These include highly pure synthetic cannabinoids as well as menthols for medicinal applications.

Picture Credits: Symrise AG

Filters enable searches by odor, stability or area of application

Design and functionality go hand in hand in the Ingredient Finder. Inviting imagery and many filter options invite users to search for the desired raw material. For example, users can search by product name, chemical name, CAS number or FEMA number.
If users are searching without that information, they can find the right product by other means. In the fragrance section, users can filter the portfolio by scents such as floral and citrus. Alternatively, users can also search for suitable applications such as soap, shampoo or lotion. Likewise, they can look for substances made from renewable raw materials or high impact substances. In the flavors section, users can search for application areas such as vanilla, tropical fruits or meat notes. They can also filter by origin, such as natural or synthetic.
"More and more consumers are looking for sustainable products," says *Antonia Lauter*, Marketing Manager for at Symrise. "As a result, manufacturers are increasingly asking for ingredients from natural and renewable sources. With the new Ingredient Finder, they can use the filter options to quickly find the aroma molecules they want."

www.symrise.com

Training for Safety Assessors of Cosmetic Products

Safety for consumers is a requirement for cosmetic manufacturers all over the world. However, the legal requirements differ. For the first time, the principle of safety assessment of cosmetic products was anchored in the European Cosmetics legislation with the Directive 93/35/EEC (6th Amendment to the EC Cosmetics Directive 76/768/EEC). Before marketing a product, manufacturers and distributors of cosmetic products are required to subject every product to a comprehensive assessment of its safety for human health. In the meantime – after the entry into force of the EC Cosmetics Regulation [Regulation (EC) No 1223/2009] in January 2010 – the requirements concerning the safety assessment have been specified in more detail (in Annex I of the Regulation).

This legal requirement of the EU serves as a model for many other regions now and there, too, safety assessment is required by law. Even in countries where safety assessment is not legally required, manufacturers want to make sure to bring safe products to market for the consumers. These manufacturers often use the requirements of the EU, although the comprehensive documentation does not necessarily have to be carried out.

EU law requires that a personally responsible safety assessor for the safety of a cosmetic product has to be appointed. A cosmetic product must meet all requirements of the EC Cosmetics Regulation in terms of its effects on human health in the event of normal or reasonably foreseeable use. When preparing a safety assessment, first of all, the expert must review all existing basic requirements of cosmetic legislation (substance regulations, labelling provisions etc.). The more extensive assessment must be in conformity with Article 10 and Annex I of the EC Cosmetics Regulation. In this connection, the toxicological profiles of all ingredients and the exposure conditions to be expected must be taken into due account.

For the elaboration of the safety assessment, the legislator has specified a certain group of individuals. Article 10 of the EC Cosmetics Regulation stipulates in Section 2: "The cosmetic product safety assessment, as set out in Part B of Annex I, shall be carried out by a person in possession of a diploma or other evidence of formal qualifications awarded on completion of a university course of theoretical and practical study in pharmacy, toxicology, medicine or a similar discipline, or a course recognized as equivalent by a member state."

However, such an education is not sufficient as a rule. For the competent assessment of the safety of cosmetic products, interdisciplinary knowledge is required, in particular in the fields of chemistry, toxicology, dermatology and (cosmetics) law so that the corresponding person must engage in specific continuing education in these fields. Together with IKW and university experts, DGK e. V. developed continuing education courses for safety assessors for the first time in 1998. The structure and the content of the courses were extensively revised and updated in 2006. More emphasis was put on practice. The lecturers for these courses are highly qualified experts from the respective disciplines from universities, public authorities and industry, including, for instance, several members of the Cosmetics Commission of the Federal Institute for Risk Assessment (BfR) or the SCCS (Scientific Committee on Consumer Safety).

The cycle of courses consists of seven individual courses of two days each. At the end of each course, a written test can be made (participation on a voluntary basis). 18 of 24 questions must be answered correctly to obtain a corresponding certificate for the attended course.

The series is divided into the following courses which can be attended in any order:

- Exposure of Cosmetic Products/Percutaneous Penetration
- Topical Safety, Immunology and Sensitisation
- Metabolism, Kinetics and Structure-Activity Relationships
- Carcinogenesis and Mutagenesis
- General and Systemic Toxicology
- Reproduction Toxicology
- Microbiological Safety of Cosmetic Products

Additionally, DGK and IKW offer two to three seminars for safety assessors per year. The session features lectures on latest developments or specific topics in the area of safety assessment. Also, participants have the opportunity to work together in small groups on practical exercises which have been provided ahead of the meeting. Meanwhile, such seminars are offered in several countries like Poland, India, South Africa etc. Some of these seminars are also offered digitally.

The courses and seminars are available in German and English. Since July 2019, the courses are also available as web trainings. It is possible to mix any number of face-to-face and web trainings to receive the certificate of successful participation in the full 7-course cycle. Trainings and web trainings are fully equivalent in this respect.

Safety assessors who meet the minimum education requirements of Article 10 of the EC Cosmetics Regulation and who have successfully completed the 7-course series of trainings, and who have participated in at least three seminars for safety assessors in the past three years, may apply for the optional certificate "DGK Safety Assessor". The certificate reflects the advanced qualifications of its holder and is valid for five years. It can be prolonged if the applicant can provide evidence for the participation in a certain number of further seminars during the past five years. Meanwhile such seminars are offered by our partners in the different regions all over the world.

This concept is unique in this form worldwide. So far, more than 200 people have completed all 7 courses, and the certificate "DGK safety assessor" has already been awarded more than 130 times.

For courses and seminars please consult
www.safetyassessor.info

Virtual Opening Event at Isobionics New Distillation Plant at Site in Geleen, Netherlands

Geleen, Netherlands | 11 February 2021. Toine Janssen, CEO of Isobionics® formally opened the new Isobionics distillation plant at Brightlands Chemelot Campus in Geleen, Netherlands. Steffen Götz, Head of BASF's Aroma Ingredients business, Jurgen Hoekstra, Managing Director BASF Nederland, and Bert Kip, CEO of Brightlands Chemelot Campus, joined the event virtually.

The Isobionics approach to producing natural fragrance and flavor ingredients is unique around the world. The company´s proprietary technology is based on fermentation, a traditional technique well known from processes such as brewing beer and baking bread. In this method, a substance is converted into a target product with the help of microorganisms such as bacteria or fungi. This process is based on renewable raw materials. Distillation is the last production step, yielding natural ingredients.

"With our new distillation plant, we can expand our business even faster and serve the needs of our customers. We have many new ingredients in development, some of them will be already launched this year," says *Toine Janssen*, CEO of Isobionics.

"The new distillation plant is another example how we connect the innovation power and scale-up capabilities of Isobionics and BASF – for one purpose: to best serve the needs of our customers," says *Steffen Götz*, Head of BASF's Aroma Ingredients business.

"Thanks to the support from the Province of Limburg and the excellent facilities available at Brightlands Chemelot, we can now expand our distillation capacity. Isobionics' new fermentation-based products highlight the innovative strength of the ecosystem in the south of the Netherlands," says *Jurgen Hoekstra*, Managing Director BASF Nederland.

The latest innovation: Isobionics® Santalol

Isobionics recently launched Isobionics Santalol. The proprietary manufacturing process makes the product unique. It is produced on a biotechnological basis from renewable raw materials: The starting material for the fermentation process is corn starch obtained from corn grown in Europe. The product is 100% free of the endangered sandalwood tree, which is on the Red List of the International Union for Conservation of Nature (IUCN). Isobionics Santalol is a close alternative to the original sandalwood oil, bringing warmth and volume to any fragrance creation while conserving natural resources. In addition, it offers consistent high quality and is independent of weather and harvesting conditions. This ensures stable product availability.

Isobionics is a leading innovator in fermentation, located in Geleen, Netherlands. The company develops and produces a wide range of ingredients for the F&F market with a focus on citrus oil components such as Valencene and Nootkatone. In 2019, Isobionics was acquired by BASF and became part of BASF's Aroma Ingredients business.

www.basf.com.

Lipoid Kosmetik AG Proudly Announces the RSPO Mass Balance Certification

Steinhausen/Switzerland, February 12, 2021. Excessive consumption of palm (kernel) oil leads to many environomental and social issues along the supply chain, hence the importance of sustainable palm oil production increases each year.

Sustainable and responsible behaviour, – based on environmental, social and economic actions – is a fundamental part of Lipoid Kosmetik's business philosophy. For that reason, we have a long history regarding the use of sustainable palm (kernel) oil and its derivatives. It has been part of our purchasing specification since years. Further, Lipoid Kosmetik is a member of RSPO since 2017.

Now we are proud to announce that we reached the next level:

Lipoid Kosmetik AG received the RSPO certification according to the RSPO Supply Chain Standard, Mass Balance. This certification confirms that we contribute to the production of sustainable palm oil by using palm oil derived from sustainable supply chains. Our RSPO certification number (RSPO 7-02527-2020) can be found on the Product Composition Sheet and the Certificate of Analysis of all concerned products.

With this certification our products claimed as RSPO MB-certified are now proven to contribute to a sustainable way of using palm (kernel) oil and are able to support final cosmetic products regarding their RSPO certification claim.

www.lipoid-kosmetik.com

index of advertisers

imprint

Organ

SEPAWA (Vereinigung der Seifen-, Parfüm- und Waschmittelfachleute e.V.)

Editor in Chief
Robert Fischer | robert.fischer@sofw.com

Editorial Content
editorial@sofw.com

Advertising
advertising@sofw.com

Subscriptions
subscription@sofw.com

Publisher
Verlag für chemische Industrie H. Ziolkowsky GmbH

Print

Holzmann Druck GmbH & Co. KG
Gewerbestraße 2 | 86825 Bad Wörishofen
Germany

Issues
10 issues per year + scheduled special issues

Address

Verlag für chemische Industrie H. Ziolkowsky GmbH
Dorfstr. 40 | 86470 Thannhausen
Germany

Phone
+49 8281 79940-0

Fax
+49 8281 79940-50

Email
vci@sofw.com

Internet
www.sofw.com

Advertisement Rates, See Media Info
www.sofw.com › SOFW Journal › Mediadata

Subscription, Prices
Print & Online Subscription
› EUR 235.00 (+ postage, + VAT where applicable)

Online Subscription
› EUR 195.00 (+ VAT where applicable)

Price/Issue
Germany: EUR 23.36 (+ postage, + VAT where applicable)
Other Countries: EUR 23.36 (+ postage, + VAT where applicable)

Picture Credits
shutterstock.com: WAYHOME studio, Prostock-studio

147th Volume: SOFW Journal Soaps, Oils, Fats, Waxes
94th Volume: Containing Sections for Cosmetics, Aerosols and Perfumery
117th Volume: International Journal for Applied Science in the Chemical Industry

Gmoist Sea-Gel-H

Gmoist Sea-Gel-H is a novel moisturizing agent derived from *Fucus vesiculosus*. It inspires moisturizing power from the ocean.

- Regulate cornified envelope from genetic expression
- Repair and strengthen skin barrier
- Long lasting moisturizing effect for 48h
- Fresh and clean skin feeling

www.greaf.com
export@greaf.com.cn
7F, Building 1, #58 Yuanmei Road, Shanghai, PRC

Any distributors are welcomed for cooperation

GREAF
Active Ingredients