» Scientific Information

Ectoin®

The natural stress-protection molecule

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1. Introduction

Extremolytes are protective small molecules from extremophilic microorganisms. bitop AG develops and markets extremolyte-based products for the treatment of respiratory diseases, allergic and dermatological conditions. ***Ectoin***® is a registered trademark of bitop AG (Witten, Germany) used for the extremolyte ectoine and ectoine-based products developed and produced by bitop. Ectoine represents a widely applicable, well-tolerated and natural protective molecule against diverse harmful environmental influences such as heat, aridity or UV radiation. The cell-protective and anti-inflammatory mode of action could be verified in various *in vitro* and *in vivo* studies.

bitop AG is the exclusive manufacturer of ***Ectoin***® in an industrial (metric ton) scale and a quality suitable for use in medical devices. bitop AG has an ISO 13485:2003 based quality management system for the development and production of extremolyte-based medical devices and ***Ectoin***®.

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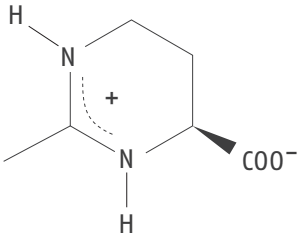
» 2. Characteristics and origin

Trade name	***Ectoin®***
Chemical name	Ectoine; (4S)-2-methyl-1,4,5,6-tetra-hydropyrimidine-4-carboxylic acid
CAS No	96702-03-3
Molecular formula	$C_6H_{10}N_2O_2$
Structure	
Molecular weight	142.16 g/mol
Melting Point	280 °C (decomposition)
Acidity/Alkalinity	pKs_1 = 2.44 pKs_2 = n.a.
Angle of rotation	$[\alpha]_D^{25}$ = 141.7°
Solubility at 25°C	Water: ~ 550 g/l Methanol: ~ 36 g/l Ethanol: ~ 5 g/l
Characteristics	Ectoine is a colourless, crystalline, slightly hygroscopic solid. It is very stable in a wide pH range (1–9) and at high temperatures (6 h at 190 °C).
Storage	For optimal shelf life to be stored tightly closed and dry at 15–25 °C Shelf life > 4 years

» *Ectoine – $C_6H_{10}N_2O_2$*

Ectoine is a low molecular, cyclic amino acid derivative, which is produced by many different extremophilic microorganisms. Ectoine belongs to the class of compatible solutes also called extremolytes (osmolytes from extremophiles) and was first isolated by Galinski and colleagues from the bacterium *Ectothiorhodospira halochloris* found in Wadi Natrun, Egypt [1].

In extremophilic microorganisms these low molecular weight compounds are accumulated in response to increased extracellular salt concentrations, but also as a response to other environmental changes, e.g. increased temperature. This is one of the ingenious strategies these organisms have developed to cope with harsh environmental conditions.

Extremolytes minimise the denaturation of biopolymers that usually occurs under conditions of water stress and are compatible with the intracellular machinery at high (>1M) concentrations. Extremolytes have a wide range of applications due to their protection of biological macromolecules and cells from damage by external stresses.

One of the first extremolytes that was produced on a large scale is ectoine (trade name ***Ectoin***®, a registered trademark of bitop AG, Germany). It is already used as a cell protectant in dermatological creams, skin care and as a stabiliser for proteins and cells in life sciences.

» 3. Mode of action

Ectoine stabilises biomolecules via a physical mechanism, called "preferential exclusion" [2]. According to this theory, the protein stabilisation effects of osmolytes like ectoine are due to their effect on the solvent water leading to a preferential exclusion of the osmolyte from the protein surface and thereby to a preferential hydration of the protein.

Because the surface area of globular proteins in the native state is smaller than in the denatured state, the equilibrium is shifted to the native state resulting in stabilisation of the native structure.

The effect is based on several mechanisms:

1. Steric exclusion from the protein surface: this plays a role only in cases where the protective molecule is substantially larger than water.

2. Increase of the surface tension of water by the protective molecule: According to the Gibbs adsorption isotherm this must result in the exclusion of ectoine from the water–macromolecule interface.

3. Preferential hydration due to the solvophobic effect: Solvophobicity is a consequence of increased hydrophobic interactions caused by a solute molecule that enforces the water structure. According to this concept of the osmophobic effect, the repulsion between the amide backbone of the protein and the osmolyte is due to the influence of the osmolyte on the water structure.

   The osmolyte promotes the formation of water molecules in clusters. Thus the exclusion hypothesis attributes the stabilising effect of ectoine to changes in the surrounding water structure.

Ectoine is in contrast to e.g. sodium chloride a strongly kosmotropic (water structure forming) substance. An investigation of the oxygen radial distribution function of pure water, sodium chloride and ectoine proved the stabilising effect of ectoine on the water structure (Figure 1).

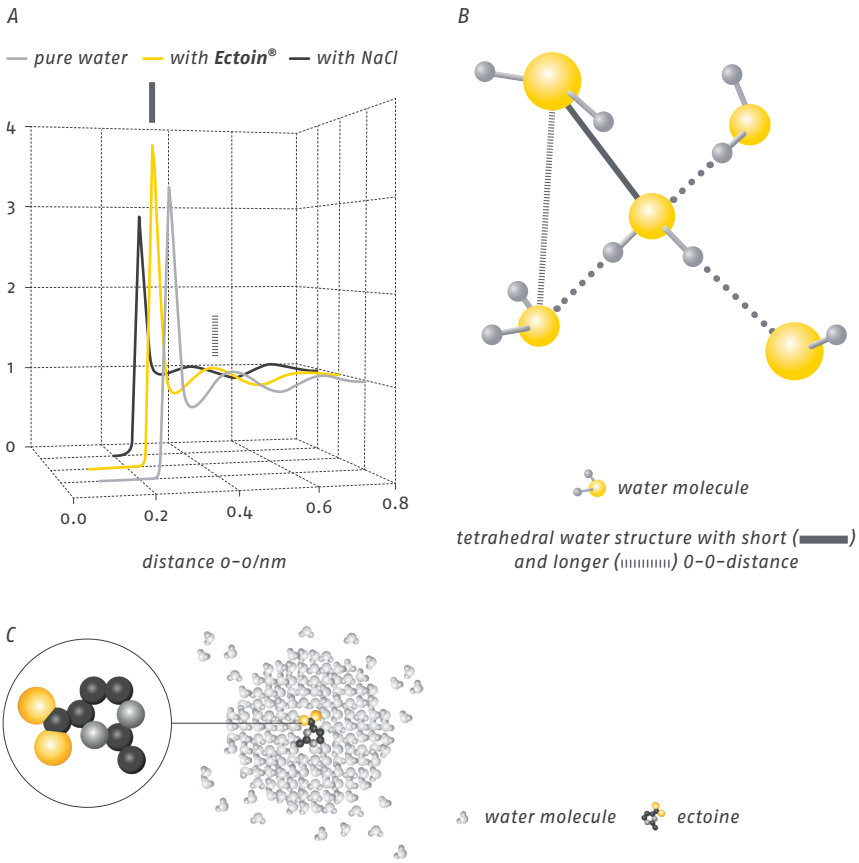


Figure 1: *Effect of ectoine on the water structure*

A) The radial distribution function of pure water (—) shows a maximum at approx. 0.3 nm (▬), which demonstrates the direct interaction of two neighbouring water molecules. The smaller maxima at 0.45 nm (⁞⁞⁞⁞⁞⁞⁞⁞) and 0.7 nm demonstrate interactions of not neighbouring water molecules. The addition of NaCl (—) to water leads to a decrease in the maxima. In contrast, the solution containing ectoine (—) increases the maxima and thus enhances the tetrahedral water structure [3].

B) Tetrahedral structure of water, showing the interactions between water molecules at 0.3 nm (▬) and 0.45 nm (⁞⁞⁞⁞⁞⁞⁞⁞) in the structure.

C) Complex formed by ectoine and water molecules.

Sodium chloride diminishes the interaction between water molecules. It destroys the water structure and is therefore chaotropic.

In contrast to that, a solution containing ectoine increases the number of neighbouring water molecules. Thus, ectoine enhances the water-water-interactions. The tetrahedral structure of water is stabilised by ectoine.

A molecular dynamic simulation gives further insight in the water structuring capabilities of ectoine [4]. In this computer simulation, water diffusion out of water spheres was limited and decreased enormously by adding ectoine molecules to the sphere. Even a 5-fold longer simulation time showed a stable water structure form attributable to ectoine properties, which is superior compared with water itself and outstanding compared with water glycerol complex.

To explain this phenomenon, the total potential energy (E_{pot}) was calculated. The E_{pot} value of the water-ectoine mixture was smaller than of the water molecules per se, indicating the strong organising and complexing properties of ectoine. Furthermore the E_{pot} value of the water ectoine sphere remained constant even throughout a longer simulation time.

These results also explain the stabilising effect of ectoine on proteins. The maintenance of the native state of a protein is a process driven by entropy, which results in the exclusion of hydrophobic moieties from contact with water. Stabilisation of the water structure leads to an increase of the hydrophobic interactions and therefore stabilises the whole protein structure.

Due to the exclusion of ectoine from the hydration shell of biopolymers, a protective and stabilising shield is shaped around those biomolecules, which is termed the ***Ectoin***® Hydro Complex (Figure 2).

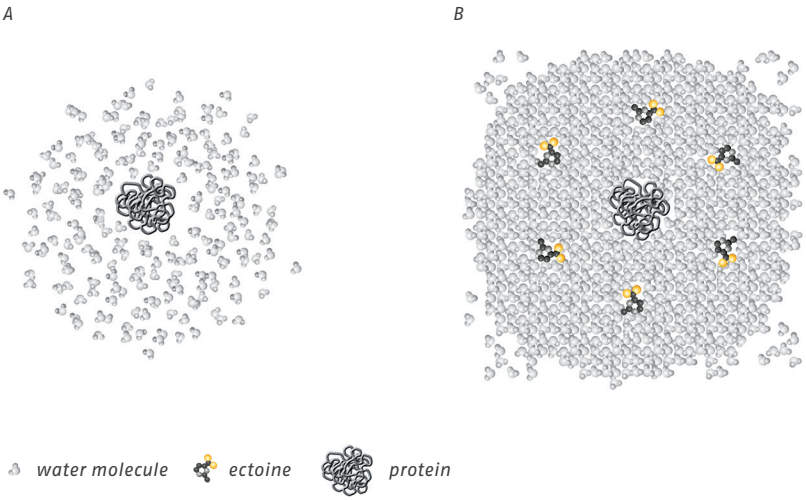


Figure 2: *Stabilisation of biomolecules via ectoine according to the preferential exclusion model*

A) Protein in water: the number of water molecules is small at the surface of the protein.

B) Protein in aqueous ectoine solution: the number of water molecules is increased by the formation of ectoine water complexes, hydrophobic interactions are increased and thus stronger stabilisation of the protein result.

The formation of ectoine water complexes and thus the kosmotropic effect of ectoine on the water structure shown above can stabilise lipid mono- and bilayers as well, which can be considered as a model for cell membranes.

As shown in Figure 3, a lipid bilayer in water is stabilised by hydrophobic interactions of the apolar lipid tail and hydrophilic interactions of the polar lipid head groups to water. In an ectoine solution, the hydrophilic interactions are increased by the ectoine water complexes resulting in increased mobility of lipids and thus fluidity of lipid bilayers.

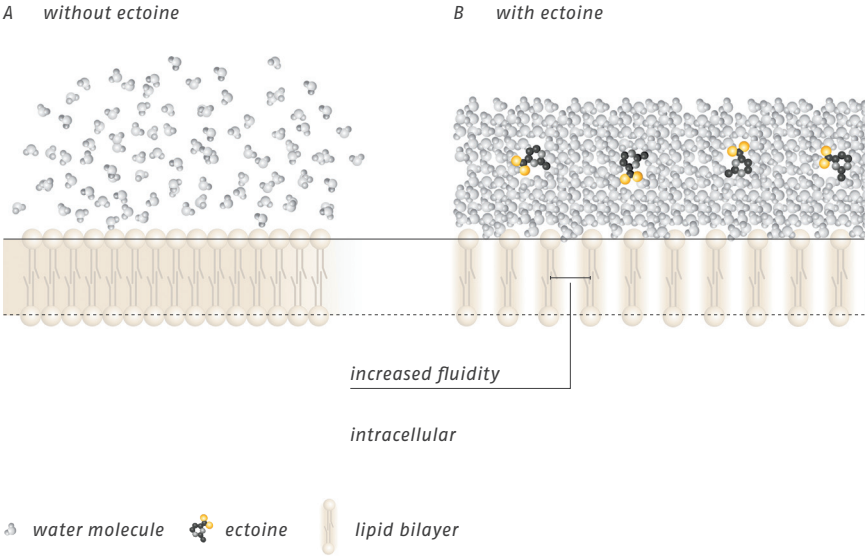


Figure 3: *Membrane stabilisation and increase in membrane fluidity due to ectoine*

A) Lipid bilayer in water: the bilayers are stabilised by hydrophilic interactions within head groups.

B) Lipid bilayer in aqueous ectoine solution: ectoine water complexes cause increased interactions of head groups with water and the membrane fluidity is increased.

The effect of ectoine on fluidity of lipid membranes was shown recently by film balance measurements of lipid monolayers (Figure 4).

By increasing the surface pressure on a DPPC lipid monolayer in water the formation of rigid well-shaped domains can be observed at higher pressure. These rigid domains are much smaller in ectoine solutions. The higher the concentration of ectoine the smaller the rigid domains. The effect is already observed at the lowest concentration tested (1 mM).

Conc. ectoine	*Surface pressure*			
	4 mN/m	*5 mN/m*	*7 mN/m*	*10 mN/m*
pure water				
1 mM				
10 mM				
100 mM				

Figure 4: *Fluorescence studies of film balance measurements*

DPPC (Dipalmitoylglycerophosphatidylcholine) Monolayer at different surface pressure and ectoine concentrations. Rigid domains appear black, fluid domains bright. Without ectoine and a surface pressure of 10 mN/m, the lipid layer exists almost completely of rigid domains. With ectoine, more fluid domains are visible. This effect increases with increasing ectoine concentration [5].

The physical state of the membrane influences the cell biology and its behaviour in response to external inputs. An increased fluidity of the membrane may for example induce the expression of stress-responsive, cell-protecting genes, such as heat-shock proteins, and reduce on-going inflammatory processes [1, 6]. The modification of the distribution of membrane proteins in a more fluid membrane also alters their activity.

For example, integrins and selectins need a specific density and length to be efficient on the adhesion of reactive leukocytes, while lipoxygenases must be bound to the membrane to catalyse the release of the pro-inflammatory signal leukotrienes. The activation of Toll-like receptors in front of signals such as lipopolysaccharide (LPS) and tumor necrosis factor (TNF)-α needs the formation of multimer complexes. Several experiments have shown that physiologically relevant fluidisation of the membrane alters their functionality [7–9].

An increase in the membrane fluidity could reduce disease symptoms and accelerate healing. It is crucial for the efficient closure of wounds [10], and it has been suggested as mechanism for the very early effects of corticosteroids in asthma therapy [11] and the beneficial effect of dietary moderate ethanol and polyunsaturated fatty acids intake in inflammatory diseases such as psoriasis, allergy, asthma and inflammatory bowel disease [12, 13].

Extracellular membrane components (transmembrane proteins, lipids, extracellular matrix) are stabilised by ectoine in their native form. Without protective mechanisms, external and internal noxa can cause increased stress for cell membranes. Cells which are in direct contact with the environment like squamous epithelial cells, i.e. skin, upper airway, lung, and intestinal tract, are particularly endangered. The external stress leads to membrane damage, which causes water loss and inflammatory reactions in the tissue.

The ***Ectoin***® Hydro Complex protects the cells against dehydration by accumulating water. Water molecules are bound more effectively near the membranes and form a stabilising and protecting complex. The impact of external pollutants on the cells is decreased by the stabilising effect of the ***Ectoin***® Hydro Complex. It protects the cells from inflammation caused by environmental stress factors like dehydration, UV radiation, tensides or airborne particles.

The ***Ectoin***® Hydro Complex protects the membrane and prevents the release of stress mediators (e.g. ceramides), which mediate inflammatory processes. Therefore inflammation can be limited (Figure 5).

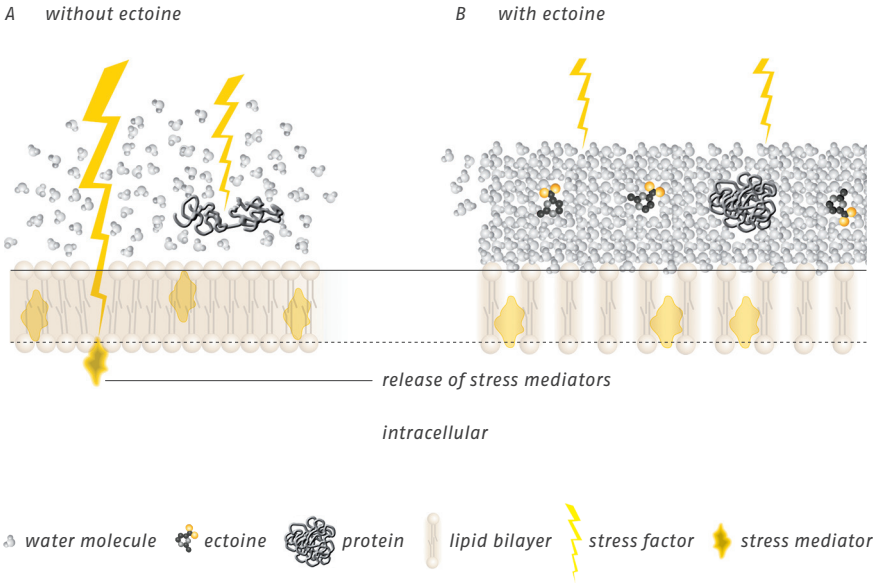


Figure 5: *Prevention of release of stress mediators (e.g. ceramides) by ectoine*

A) Without ectoine: external stress factors (like UV irradiation) cause membrane damage, water loss and release of stress mediators, which act as second messengers for inflammatory reactions.

*B) With ectoine: the **Ectoin**® Hydro Complex protects the membrane against external stress factors; stress mediator release is prevented.*

The general protective and hydrating property of ectoine against various external stress factors is the basic mechanism of our medical device developments.

4. Effectiveness and efficacy

4.1 Model systems

Various experiments with model systems or cell cultures demonstrate the protective and stabilising effect of the ***Ectoin***® Hydro Complex.

4.1.1 Protection of lipid membranes

Cell membranes are lipid double layers with integrated proteins and surface proteins. They possess specific ion channels, transport systems and receptors responsible for the signal transduction.The viability of the cells is highly associated with the efficiency of these systems. Many external factors, like temperature, radicals, pH, UV radiation, and tensides, can disturb the balance of the systems and damage the membrane.

In a red blood cell assay, ectoine showed significant protective and stabilising effects on cell membranes [14]. This assay investigates the denaturising nature of different substances on erythrocytes. Membrane damage leads to a release of the haemoglobin and thus to a red colour in the surrounding media, which can be measured photometrically. Erythrocytes have been pretreated with 1% ectoine or lecithin as positive control for one hour and stressed with different tensides.

Ectoine protects cell membranes against all kind of damaging tensides used in this test. It is even more efficient than the positive control lecithin (0-phosphatidylcholine), whose stabilising properties are well known (Figure 6).

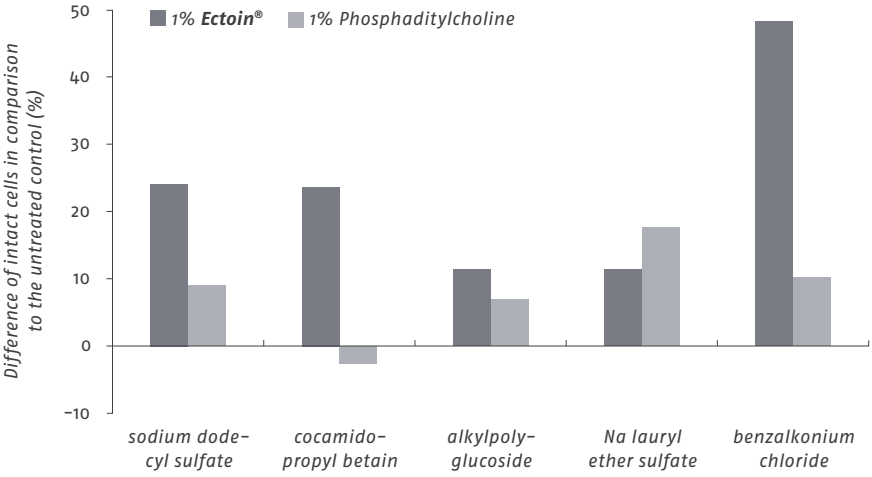


Figure 6: *Membrane stabilising effect of ectoine*

Protective effect of ectoine on the membrane of human erythrocytes against sodium dodecyl sulfate (H_{50} = 29.63 ppm), cocamidopropyl betain (H_{50} = 41.45 ppm),

alkylpolyglucoside (H_{50} = 131.76 ppm), sodium lauryl ether sulfate (H_{50} = 26.45 ppm) and benzalkonium chloride (H_{50} = 35.49 ppm). The figure shows the relative difference of cell lysis as a function of the concentration of pretreated ectoine against an untreated control in per cent. The untreated control was determined as 0. The assay was performed 5 times [15].

Graf and colleagues investigated the concentration and time dependency of this membrane stabilising effect of ***Ectoin***® [4]. The higher the ectoine concentration the greater the protective effect against membrane damage. Prolonged incubation resulted in an increase in membrane stability of 30% after 6 hours and 60% after 24 hours. Thus, the longer the cells are pretreated with ectoine the greater the protective effect.

» *4.1.2 Decrease of inflammation process caused by external pollutants*

The stabilising ***Ectoin***® Hydro Complex also decreases inflammation processes caused by external pollutants like UV radiation [14, 16, 17]. The UVA-induced signal transduction is triggered by release of ceramides (second messenger) in the cell membrane and can be measured by activation of AP-2 and generation of ICAM-1 (Figure 7).

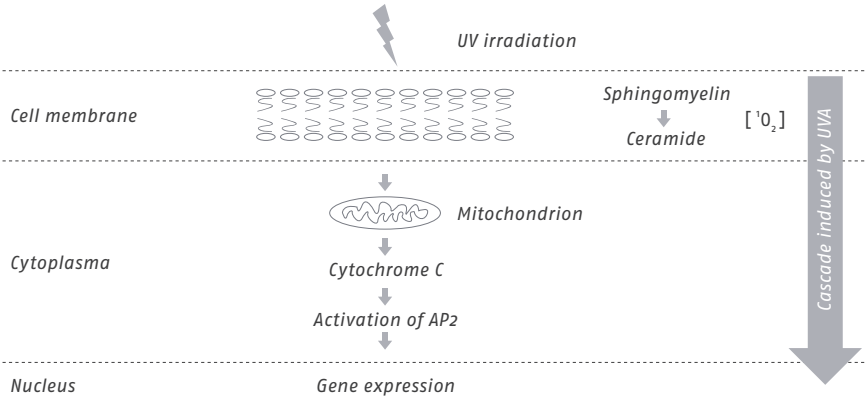


Figure 7: *Model of UVA-induced reactions in human keratinocytes modified* [17]

Human keratinocytes were pretreated with 1 mM ectoine for 24 hours and stressed via UVA radiation (30 J/cm²). The release of certain inflammation factors (AP-2, ICAM-1, ceramides) was measured. Pretreatment of human keratinocytes with ectoine leads to a significant decrease of second messenger release (Figure 8), AP-2 activation and ICAM-1 expression. Thus the ***Ectoin***® Hydro Complex diminishes inflammatory processes caused by UVA radiation. Ceramide release is a general mechanism in inflammatory processes and is also postulated for e.g. lung cells.

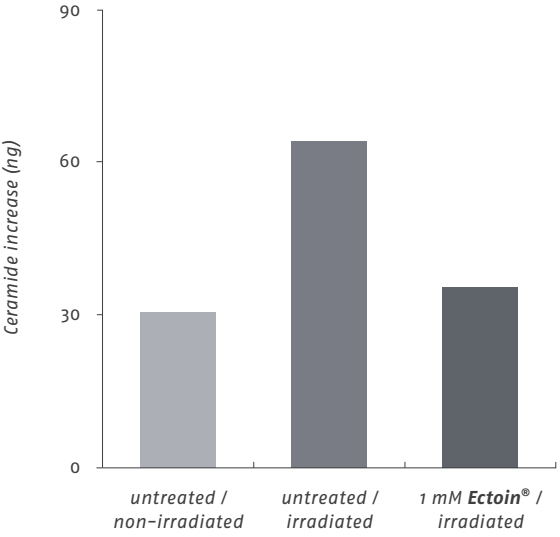


Figure 8: *Effect of ectoine on second messenger release*

UVA-induced ceramide release (ceramide split off membrane sphingomyelin) in ng. Cells were either pretreated with 1 mM ectoine or untreated. The experiment was repeated 3 times. Ectoine reduces the ceramide release after UVA irradiation [14].

4.2 Ectoine for dermatology

4.2.1 Protection against UV

UV radiation and oxidative stress is capable in generating mutations in both cellular and mitochondrial DNA. This can lead to apoptosis of the cells, reduced viability or even induce cancer. In an UV-stress model the protective effect of ectoine on the DNA of keratinocytes was investigated. Human keratinocytes were preincubated with 0.014% ectoine solution and irradiated with 5 J/cm² UVA. The UVA-induced DNA damage was evaluated by a Comet assay.

Pretreatment with ectoine solution reduces the extent of UVA-induced DNA damage [18]. Ectoine protects skin cells against damages caused by UVA irradiation.

The spherical mitochondrial DNA is located near the inner mitochondrial membrane and can be subject to increased external stress, which can lead to mutation of mitochondrial DNA. Therefore the protective effect of ectoine against mitochondrial DNA damage was investigated. Human fibroblasts were pretreated with 1 mM ectoine for 24 hours and stressed with 8 J/cm² UVA 3 times a day on 4 consecutive days over 3 weeks. The common deletions in the mitochondrial DNA were measured via PCR.

Pretreatment of keratinocytes with 1 mM ectoine was sufficient to completely inhibit UVA radiation-induced mitochondrial DNA mutagenesis.

UV radiation can damage whole cells as well as proteins and nucleic acids either directly or via formation of radicals. Once the extent of the damage exceeds a certain limit, cell death or apoptosis occurs. Keratinocytes with special characteristics, the so-called sunburn cells, are formed in the human epidermis as a result of UV radiation. It was investigated whether pretreatment (24 hours) with a 4% ***Ectoin***® formulation has an inhibitory effect on UV-induced sunburn cells in organo-typical skin equivalents (Skinethic®).

It was shown that there is a distinct relationship between the administrated irradiation dose and the number of sunburn cells [15]. Pretreatment with the ***Ectoin***® cream reduces the number of sunburn cells compared to treatment with placebo.

» 4.2.2 Protection of the immune system of the skin

Langerhans cells are one of the key components for the immune system of human skin. They are very sensitive against external stress factors, like UV stress. Therefore an effective protection of these cells is extremely important. The forearm of each participant was either pretreated with ***Ectoin***® cream or placebo formulation and irradiated with UV after 14 weeks. The number of Langerhans cells was determined [15]. Under the influence of UV radiation a significant reduction in the number of Langerhans cells (~50%) was achieved. Pretreatment with placebo led to no significant protection against UV-induced reduction of Langerhans cells. In contrast, pretreatment with a 0.3% and 0.5% ***Ectoin***® cream resulted in high and significant protection against the reduction of the number of Langerhans cells.

» 4.2.3 Protection of the human skin barrier

To show the protection of the human skin barrier function by ***Ectoin***® creams the influence of different ***Ectoin***® containing formulations on the transepidermal water loss (TEWL) was investigated. Five independent studies with various ***Ectoin***® concentrations (1–5%) and altogether over 70 patients were carried out. Transepidermal water loss in humans with sensitive skin and with atopic skin was significantly reduced due to treatment with ***Ectoin***® cream formulation.

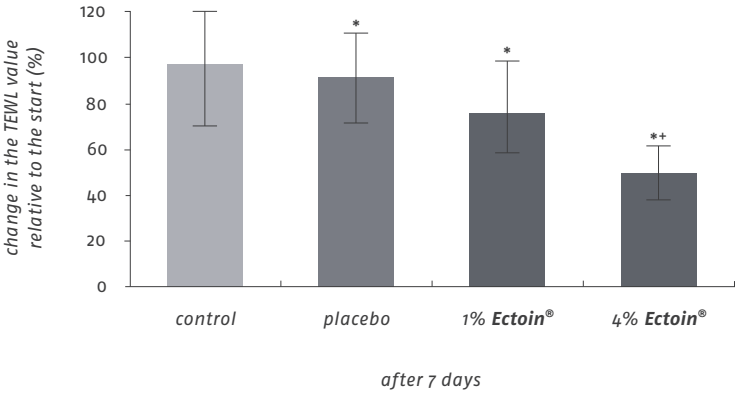


Figure 9: *Influence of* ***Ectoin***® *on the transepidermal water loss (TEWL)*

The skin on the forearm of 20 volunteers was irritated with Sodium Dodecyl Sulfate (SDS). The TEWL was measured by Tewameter. After the first measurement (t_0), the test

*products were randomly applied on the forearm twice a day for 7 days, one area remained untreated as control. The figure shows the extent of the TEWL relative to the start value (t_0); *p<0.05 versus untreated; +p<0.05 versus untreated, placebo.*

4.2.4 Improvement of overall skin condition

The influence of the ***Ectoin***® Hydro Complex on skin roughness and scaliness was investigated in three independent studies with different ***Ectoin***® concentrations (2% and 5% ***Ectoin***®). ***Ectoin***® reduced skin roughness up to 86% after 3 to 4 weeks of treatment and skin scaliness up to 70% after 3 to 4 weeks of treatment. Additionally, the skin of 20 test persons was stressed with SDS and the erythema was measured. After 7 days of treatment with placebo and ***Ectoin***® cream formulation, respectively, the reduction of the erythema was determined (Figure 10).

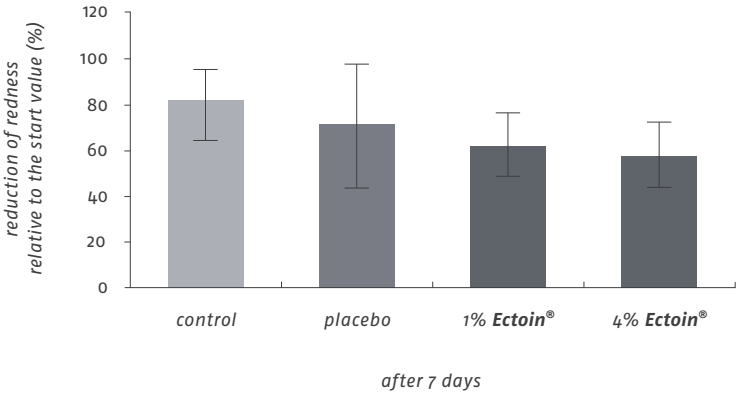


Figure 10: *Reduction of erythema after 7 days of* ***Ectoin***® *treatment*

Erythema was induced on the forearm of 20 volunteers via SDS-patch assay and determined by chromametry. After the first measurement (t_0), the test products were randomly applied on the forearm twice a day for 7 days, one area remained untreated as control. The figure shows the extent of the erythema relative to the start value (t_0).

The erythema was reduced by up to 23% after 7 days of treatment with a 4% ***Ectoin***® cream.

4.2.5 Clinical evaluation: psoriasis

Another observational study with 94 patients showed a good efficacy of a 5% ***Ectoin***® cream formulation for the treatment of psoriasis [19]. The efficacy has been assessed as very good or good on the part of the physicians and the patients in more than half of the cases. The symptoms dryness, scaliness, and pruritus decreased (Figure 11).

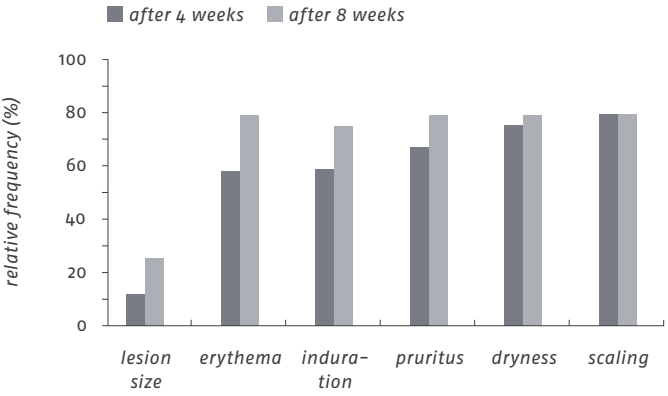


Figure 11: *Improvement of the symptoms of psoriasis*

94 patients with chronic plaque psoriasis were treated with 5% Ectoin® cream for about 8 weeks. At the beginning, after about 4 weeks, and after about 8 weeks the symptoms were rated on a 1–7 scale (none to severe) by the physician. The figure shows the improvement rates of the lesional areas in patients with complete observation (N=24).

4.3 Long-term moisturising effect of ectoine

4.3.1 Enhancement in skin hydration

The human skin and other epithelia like the nasal mucosa and the eye epithelia have complex epidermal fluid regulation systems. The balance of these systems can be easily disturbed by external stress factors, like tensides, high or low temperature, or low humidity. These lead to dry skin or mucosa. It was shown that ***Ectoin***® increases the skin hydration by up to 39%. The results also show that

Ectoin® maintains a considerably greater degree of skin moisture than untreated or placebo-treated skin, even after 24 hours. ***Ectoin***® protects skin against rapid dehydration after direct application of hygroscopic silica gel. Skin moisture can be maintained for a longer period of time by topically applying ***Ectoin***® [4].

In a further series of experiments, the long-term moisturising effect of ***Ectoin***® was evaluated. The test was carried out on the forearm of volunteers. 0.5% and 1% ***Ectoin***® were applied twice a day for 12 days. The skin hydration was measured from day 8 to day 12. On day 12, the application of ***Ectoin***® was stopped for 7 days, detecting the skin hydration finally at day 19. After 8 days of application, the hydration increased markedly up to 200% compared with the placebo-treated skin and remained constant until the end of the testing period. Although the topical application was stopped on day 12, the actual hydration status was preserved for further 7 days underlining a significant long-term moisturising effect of ***Ectoin***® (Figure 12).

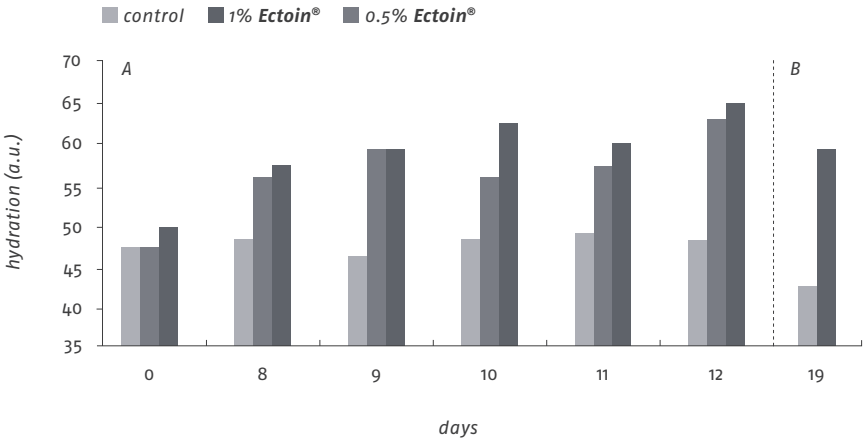


Figure 12: *Hydrating effect of* ***Ectoin***® *Hydro Complex on the skin*

A) The skin of the forearm of 5 volunteers is treated with a formulation containing 0, 0.5% or 1% ***Ectoin***® *twice daily for 12 days. On day 8 to day 12, the skin hydration is determined by corneometry*

B) After day 12, the treatment is stopped and the skin hydration is measured on day 19.

Ectoin® *has a long lasting moisturising effect on skin cells* [4].

4.4 Ectoine for inhalation to treat symptoms of respiratory impairment

4.4.1 Protection against nanoparticle-induced neutrophilic lung inflammation

Nanoparticles of occupational and environmental origin (particulate matter: PM) can induce inflammatory reactions of the airways. The effects of inhaling PM have been widely studied in humans and animals and include asthma, COPD, lung cancer, cardiovascular issues, and premature death [20]. The size of the particle is a main determinant of where in the respiratory tract the particle will come to rest when inhaled. The notation $PM_{2.5}$ is used to describe particles of 2.5 µm or less.

Because of the size of the particle, it can penetrate into the deepest parts of the lung. As this kind of exposure in industrial societies is often unavoidable, a strategy of prevention on an individual level is necessary to prevent inflammation-derived secondary diseases.

Because the ***Ectoin***® Hydro Complex has proven to reduce cell stress effects, it was investigated whether it is capable to prevent nanoparticle-induced lung inflammation [21].

Ectoine, either given together with or prior to the nanoparticles, dose-dependently reduced neutrophil inflammation in the lung [21]. Ectoine administration inhibited nanoparticle-induced signalling known to be responsible for pro-inflammatory reactions in rat lung epithelial cells *in vitro* as well as *in vivo*.

Animals pretreated intratracheally with ectoine solution before PM instillation show reduced IL-8 expression, reduced neutrophils in the lung, cytokine profile modulation and reduced MAP-kinase activation (Figure 13).

These findings were corroborated and extended in experiments with cultured human bronchial epithelial cells in which ectoine inhibited nanoparticle-triggered cell signalling and IL-8 induction.

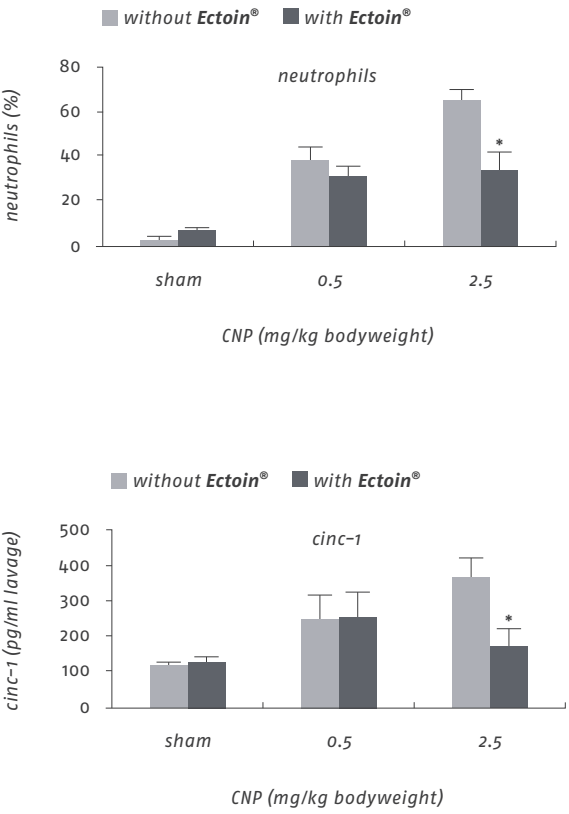


Figure 13: *Protective effect of ectoine against nanoparticle-induced lung inflammation*

*Ectoine reduces dose-dependently carbon nanoparticle (CNP)-induced inflammation **in vivo**. Bronchoalveolar lavage parameters (% neutrophils and cinc-1) from Fischer 344 rats intratracheally instilled with 0.4 mL CNP in the presence or absence of ectoine are shown. Ectoine effects on different doses of CNP after 48 hours. Animals (n = 5) were instilled once with phosphate-buffered saline (PBS) (sham) or with 0.5 or 2.5 mg/kg body weight CNP alone (solid bars). Shaded bars indicate an instillation of CNP together with 1 mM ectoine.*

4.4.2 Protection against induced asthmatic reaction

There is a rapid increase in allergic asthma and other atopic disorders in industrialised nations. The protecting film formed by the ***Ectoin***® Hydro Complex could prevent the contact of allergens with the cells and thus help to minimise inflammatory reactions driven by allergens.

It was investigated whether a intratracheal treatment with ectoine has a protective effect on the experimentally allergen-induced early response (EAR), late airway hyperresponsiveness (AHR) and inflammation in the sensitised Brown Norway rat as a model of allergic asthma in humans [22]. Results of the studies are shown in Figure 14 and Figure 15.

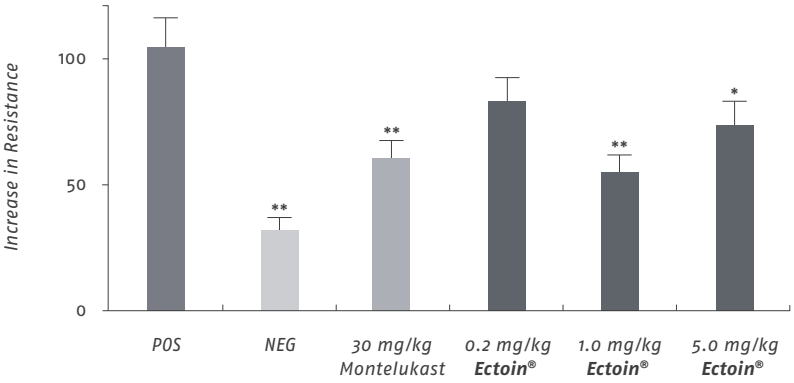


Figure 14: *Lung function before and during ovalbumin challenge (early allergic response)*

*Maximum increase in resistance above baseline values prior to provocation (*p<0.05; **p<0.01). Ectoine has a positive influence on the increase of resistance caused by the ovalbumin challenge and reduces the increase in resistance significantly compared to the positive control group and comparable to the control medication Montelukast (n>16 per group).*

Ectoine showed marked and significant therapeutic effects on the EAR, AHR and inflammatory response in this animal model of asthma. In comparison, ectoine showed the same or a slightly increased inhibition as montelukast at only 1/30 or 1/6 of the montelukast dose, respectively (at 1 or 5 mg/kg).

These findings support a potential prophylactic and therapeutic usefulness of inhaled ectoine in allergic and/or asthmatic conditions of the respiratory tract.

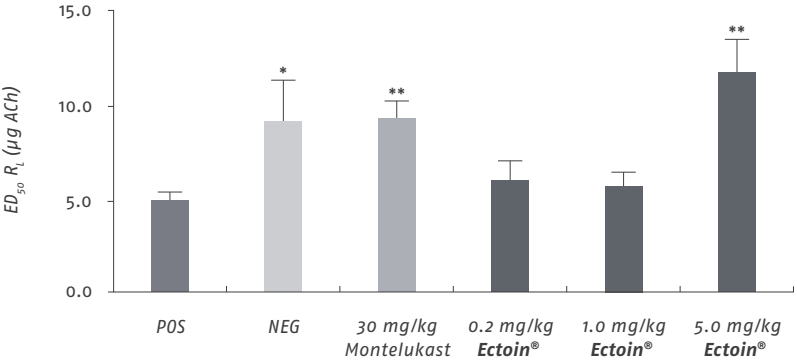


Figure 15: *Hyperresponsiveness test in the late allergic phase (24 hours after ovalbumin challenge)*

*inhalational ACh dose (µg) to produce a 50% increase in resistance (ED_{50}) (*p<0.05; **p<0.01). The effective dose (ED_{50}; ED_{100}; inhalational ACh dose) required to increase the lung resistance by 50% can be increased by ectoine (n>16 per group).*

» 5. Safety and tolerability

5.1 Toxicologic data

Several studies regarding the toxicologic capability of ectoine have been made. The investigations were performed according to different international approved guidelines (OECD or ISO). In summary, ectoine has no toxicological potential in the tested concentrations applicable for a use in humans.

5.2 Adverse effects

At present, no adverse effects have been observed in all different clinical trials. Even sensitive persons like patients with atopic dermatitis reported no negative side effects. ***Ectoin***® has been used in the cosmetic and healthcare industry for many years. This wide-spread use by consumers in skin care applications also revealed no adverse effects.

5.3 Precautions

Pregnancy and nursing mothers

No studies have been done on the safety and effectiveness of this device in pregnant women or nursing mothers. It is not known whether ectoine is excreted in human milk, although the existing studies indicate it as unlikely. Nevertheless, caution should be exercised when ectoine is administered in nursing women.

Pediatric Use

Safety and effectiveness of ectoine in pediatric patients have not been established. Skin care products (e.g. sun care products) for children containing ectoine have been marketed without reports of adverse effects.

Hypersensitivity

At present, no hypersensitivity against ectoine has been observed. Still it is possible that hypersensitivity occurs. Thus, ectoine is contraindicated in patients with known hypersensitivity against ectoine.

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