

# Abstracts

26. Jahrestagung

Weitere Poster-Abstracts



Gesellschaft für  
Dermopharmazie

# Influence of Ethanol as a Preservative in Topical Formulation on the Dermal Penetration Efficacy of Active Compounds in Healthy and Barrier-Disrupted Skin

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Ethanol (EtOH) is a multifunctional excipient often used as a preservative in topical formulations. When added in concentrations above 15% to the water phase, it acts as a natural preservative and is considered to be safe and non-toxic [1]. EtOH is also known to act as a solvent and penetration enhancer and can impair skin barrier function [2]. This study investigated EtOH as a preservative in a commercially available oil-in-water cream formulation to evaluate its effect on bio-physical skin properties and dermal penetration [3].

Bio-physical skin properties, including transepidermal water loss (TEWL) and skin hydration, were measured with probes from Courage & Khazaka Electronic GmbH (Cologne, Germany) using an ex vivo porcine ear model. Skin barrier function was evaluated on healthy, intact skin and irritated skin mimicking barrier-disrupted conditions [4]. Additionally, dermal penetration efficacy was assessed by cryosectioning skin biopsies, followed by inverted epifluorescence microscopy and digital image analysis [5].

When EtOH was added to the cream, the increase in skin hydration on intact skin was less pronounced than with the cream without EtOH, confirming the skin-dehydrating effect of EtOH (Fig. 1) [3]. On irritated skin, however, the cream with EtOH did not cause a dehydrating effect but showed a slight increase in skin hydration instead. These findings are in line with the TEWL data, which indicated a dehydrating and barrier-disrupting effect on intact skin, but no irritating effects on already irritated skin. The results demonstrate that skin impairment can be considered to have different stages, while in an early stage, the formation of a "Pudding skin" is proposed. The "Pudding skin" is a thin layer of dried skin on top that "seals" the lower parts of the skin, reduces water loss from inside, and limits the dermal penetration of chemical compounds from outside the skin.

Differentiating between skin conditions is essential in understanding how EtOH, as a preservative, influences topical formulations, particularly in products for sensitive or barrier-disrupted skin. The



choice and concentration of preservatives, along with overall formulation composition, are crucial to effectiveness and require careful customization to optimize therapeutic outcomes across diverse skin types.

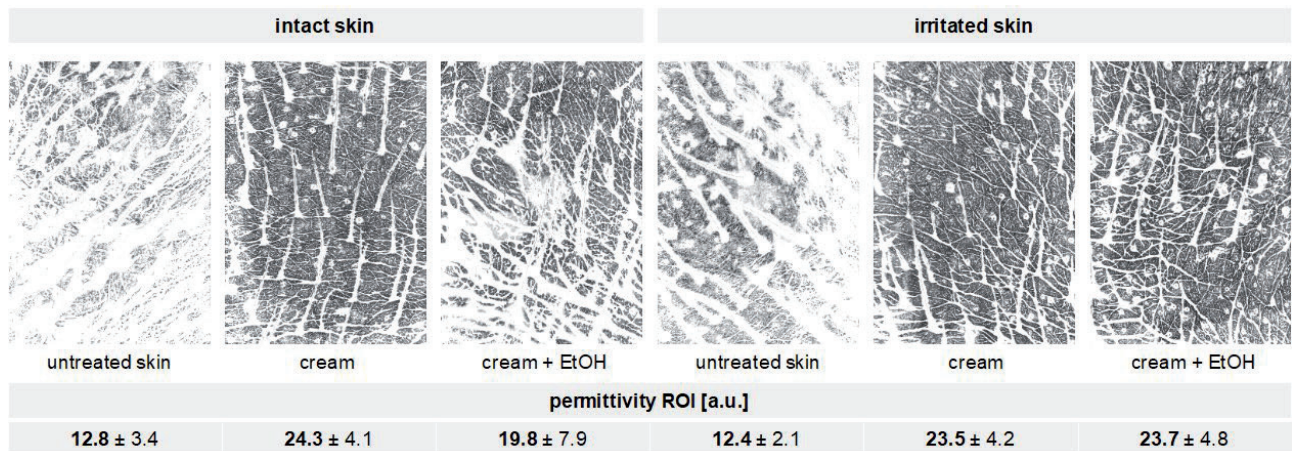


Figure 1: Influence of skin treatment with cream and cream preserved with EtOH (20% v/v) on moisture distribution (MoistureMap MM200) of intact and irritated skin. Permittivity values are shown below as mean ± standard deviation. [3]

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# Topical formulations and their dual effect on skin hydration and stratum corneum thickness

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**Introduction:** Skin hydration is an important parameter for skin health and well-being [1]. Therefore, in skin formulation development, it is also an important parameter to test whether a formulation provides good or relatively poor skin hydration properties. Skin hydration can be measured in vivo using skin probes that measure the capacitance of the skin [2]. However, these skin probes can only measure the conductivity at the surface of the skin. Hence, it is hypothesized that the measurements can have bias, for example due to remaining formulations on top of the skin that hold water and/or form films which hinder direct contact of the skin probe with the skin. Consequently, the measurement may reflect the water content in the formulation on top of the skin rather than the water content of the skin itself. Sweat or massage that squeezes water from the skin can also introduce artifacts, leading to inflated skin hydration measurements. An additional method to measure skin hydration is the ex vivo measurement of stratum corneum thickness (SCT). A thicker stratum corneum represents greater skin hydration and vice versa [3].

**Aim:** The aim of this study was to assess surface skin hydration from differently treated skin areas with in vivo skin probes and to compare the results with SCT measurements.

**Materials and Methods:** Different formulations were prepared. A hydrogel served as a base in which different types of particles were added. The particles differed in size and type of surface coating. The formulations were applied to ex vivo skin (fresh porcine ears) and the surface skin hydration (measured with a Corneometer® CM 825 and the MoistureMap MM 200, Courage+Khazaka electronic GmbH, Cologne, Germany) was assessed 1 hour after application, immediately after the formulations were gently wiped off with a soft tissue. SCT was assessed by analysing 20 µm cryosections from skin biopsies taken from the different skin areas using inverted epifluorescence microscopy and digital image-analysis software. The results obtained from the different skin hydration measurements were compared and correlated using JASP software [4].

**Results:** Skin hydration measurements showed significant differences in skin hydration for the differently treated skin areas. However, the trends seen between the different skin hydration parameters were not correlated with each other. Thus, surface parameters yielded different trends than SCT parameters, and even the surface parameters did not always show the same trend. The



results confirm the hypothesis that SCT and surface hydration measurements are not identical. Each method yields valuable results but answers different questions. Surface measurements assess hydration at the surface of the skin. Corneometer measurements sample small areas, and the MoistureMap measures larger areas and thus provides a broader view of the distribution of water within the measured skin area. SCT measures hydration within the skin, particularly within the stratum corneum. The results provide evidence that formulations that effectively hydrate the SC do not necessarily hydrate the surface of the skin. A prominent example in this study is particles added to the gel: they decreased surface skin hydration but did not alter inner skin hydration (i.e., SCT).

**Conclusions:** Surface skin hydration measurements alone cannot provide absolute information about the skin hydration potential of a formulation. Additional measurements are suggested to understand and judge a formulation's ability to hydrate the skin more holistically.

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## 26. Jahrestagung in Tübingen: Poster-Abstracts

# Exosome-like vesicles via small-scale bead milling for improved dermal drug delivery

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Cucumber PlantCrystals (PCs) prepared by high-pressure homogenization (HPH) have been reported to enhance dermal penetration of active ingredient (AI) due to the ability of HPH to simultaneously extract and load exosome-like vesicles (EVs) from cucumbers [1]. Such ability of small-scale bead milling (SSBM) is not yet known and is therefore explored in this study.

Cucumber PCs were prepared by SSBM using Nile red (NR, 0.005% w/w) as a lipophilic AI surrogate. Solution of NR (0.005% w/w) in medium chain triglycerides (MCT) was used as control. NR containing PCs and control were applied to an ex-vivo porcine ear skin model to determine total amount penetrated (TAP) and mean penetration depth (MPD) of NR [2].

Results showed a significantly higher TAP (Figure 1A) as well as MPD (Figure 1B) for NR from PCs as compared to control. The improved dermal penetration of NR from PCs could be attributed to the NR being loaded into the lipid bi-layers of the EVs. The NR-loaded EVs in PCs formulation, thus creates a possibility for NR to take hydrophilic route of dermal penetration which could otherwise take only the lipophilic route [3]. These results demonstrate the ability of SSBM to simultaneously extract and load EVs (with AI) from cucumbers as indicated by improved dermal bioavailability. Further studies are needed to confirm the EVs based mechanism of dermal penetration enhancement by PCs.

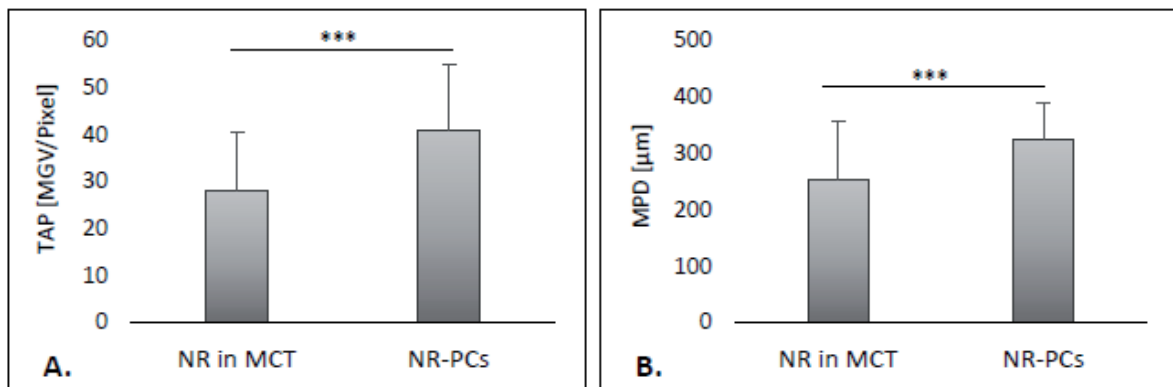


Figure 1: Total amount penetrated (TAP), B. Mean penetration depth (MPD) of NR from PlantCrystals in comparison to control (\*\*\*)  $p < 0.001$



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- [1] Abraham, A. M., et. al. (2022). Cucumber-derived exosome-like vesicles and plantcrystals for improved dermal drug delivery. *Pharmaceutics*, 14(3), 476. <https://doi.org/10.3390/pharmaceutics14030476>
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26. Jahrestagung in Tübingen: Poster-Abstracts

# Mechanism of enhanced dermal drug delivery by PlantCrystals

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Recent studies have proposed that small-scale bead milling (SSBM) to produce PlantCrystals (PCs), is capable of extracting and loading plant derived exosome-like vesicles (EVs) with active ingredients (AIs). Thus, increasing dermal bioavailability of PCs. Besides, surfactant micelles in PCs might be exerting this effect [1, 2]. The exact mechanism of PCs efficiency is unknown and is therefore investigated in this study.

PCs from cucumber were prepared via SSBM with and without surfactant, using Nile red (NR) as a lipophilic AI surrogate, and applied to ex-vivo porcine ear skin model to determine dermal penetration efficacy of NR [3]. Solution of NR (0.005% w/w) in medium chain triglyceride (MCT) and NR (0.005% w/w) loaded micelles were used as controls.

Results (Figure 1) exhibited a significantly higher dermal penetration efficacy for NR-loaded PCs, both with and without surfactant, compared to both controls. This implies that the PCs formulations exhibit an improved dermal penetration via the AI-loaded EVs as only surfactant micelles are not able to attain such improved dermal penetration efficacy. Also, the PCs prepared without using surfactant still showed enhanced dermal penetration of AI which suggests a more complex mechanism than micellar solubilization. The results therefore confirm that the mechanism of enhanced dermal penetration of AI from PCs is the loading of AI into EVs during SSBM, which further facilitates penetration of AI into the skin and leads to improved dermal bioavailability.

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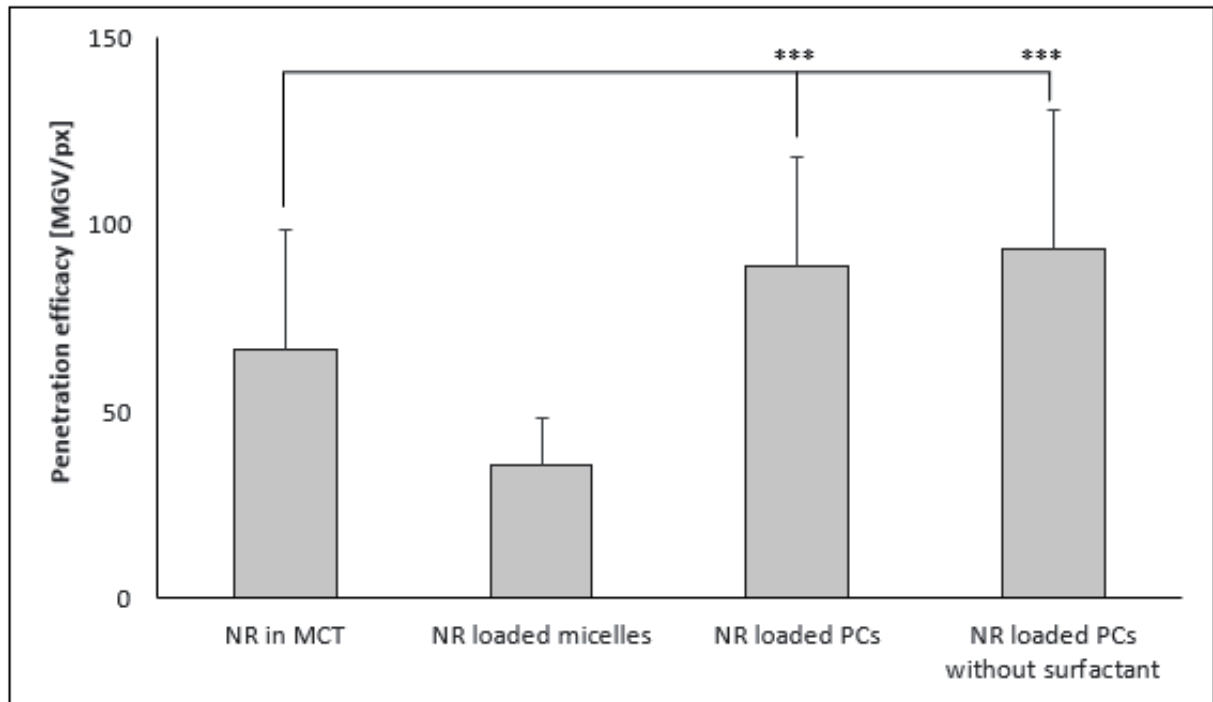


Figure 1: Ex-vivo dermal penetration efficacy of NR from PlantCrystals in comparison to controls (\*\*\*)  $p < 0.001$ )

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# Surface coating of functional particles to improve skin compatibility in topical formulations

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Drug carriers and functional particles are important excipients in topical formulations [1]. However, some of them may cause skin dehydration and alter the skin surface pH [2]. To address these effects, this study investigates the influence of surface-coating of functional particles on skin hydration and skin pH.

Two particle size fractions ( $d_{50} = 12 \mu\text{m}$  and  $44 \mu\text{m}$ ) of excipient material were coated with chitosan. Coating was performed by centrifugation to remove the solvent. Coated particles were suspended in a 1% (w/w) hydroxyethyl cellulose (HEC) gel. Using an ex vivo porcine ear model, skin hydration and pH were evaluated with three probes from Courage + Khazaka electronic GmbH (Cologne, Germany): the MoistureMap MM200 for high-resolution hydration mapping, the Corneometer® for point-specific hydration, and the Skin-pH-Meter for surface pH assessment [3].

Application of uncoated excipient particles led to a significant reduction in skin hydration (Fig. 1). In contrast, all coated formulations showed an overall positive effect on skin hydration. Particles coated with chitosan significantly improved skin hydration compared to uncoated controls and maintained levels comparable to gel-treated skin (Fig. 1). However, Chitosan-coated particles did not preserve skin pH at levels similar to untreated skin, indicating potential disturbance of the skin's acid mantle.

Taken together, these results show that coating excipient particles with skin compatible films leads to measurable improvements in skin hydration. Combining different coating agents may further enhance this effect. However, pH stabilization remained limited, suggesting that thicker or multilayer coatings could offer additional benefits. Overall, this study demonstrates that potentially irritating excipients can be converted into skin-compatible formulations using simple surface modification techniques.

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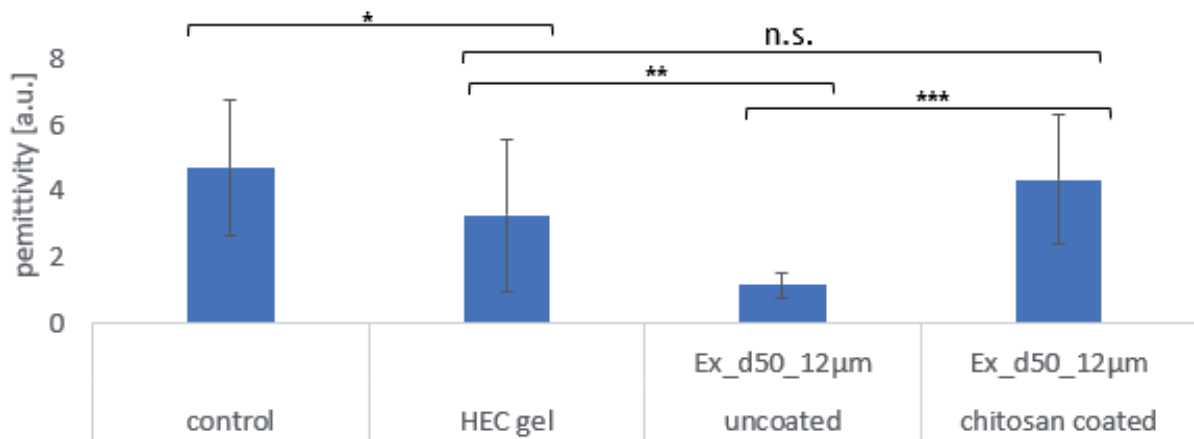


Fig. 1: skin hydration levels of untreated skin compared to skin treated with HEC gel, uncoated excipient and chitosan coated excipient.

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# Silymarin-based exosome-like vesicles and PlantCrystals to augment the dermal drug delivery

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PlantCrystals (PC) produced by high-pressure homogenization (HPH) or wet bead milling (WBM) are a proficient strategy to address formulation challenges, like dermal permeation [1,2]. These methods appear to co-generate plant exosome-like vesicles (PEVs), thereby offering a scalable substitute to low-yield ultracentrifugation [3]. This study formulates and assesses classical PEVs and PC-PEVs from Silymarin-rich milk thistle seeds.

The classical PEVs and PC-PEVs, produced by ultracentrifugation and WBM (with Tween 80 (1 % w/w), respectively [1, 2], were evaluated for kinetic dermal penetration (24 h) of Nile red (NR) using an ex vivo porcine ear model [4]. Full-thickness skin punches were placed in the 24-well plate, and treated with i) NR solution (0.005% w/w in Miglyol® 812), ii) NR-loaded classical PEVs, iii) NR-added PC-PEVs, iv) NR-loaded PC-PEVs. Fluorescence intensity was measured in a microplate reader at 32 °C for 24 h [2, 4].

Results (Figure 1) indicate that after 24 h, NR-loaded PC-PEVs achieved prominent dermal penetration, which can be attributed to active incorporation of NR into the nanosized PC-PEVs via the milling process, enabling passage through the lipid pathway. Particle-assisted penetration may further enhance permeation due to dispersion medium evaporation and aqueous meniscus formation, thus creating a higher concentration gradient that promotes AI diffusion. In conclusion, the Silymarin-based PC-PEVs, produced by simple yet effective WBM, have proven to be an excellent nanocarrier for enhanced penetration efficacy of the lipophilic drug.

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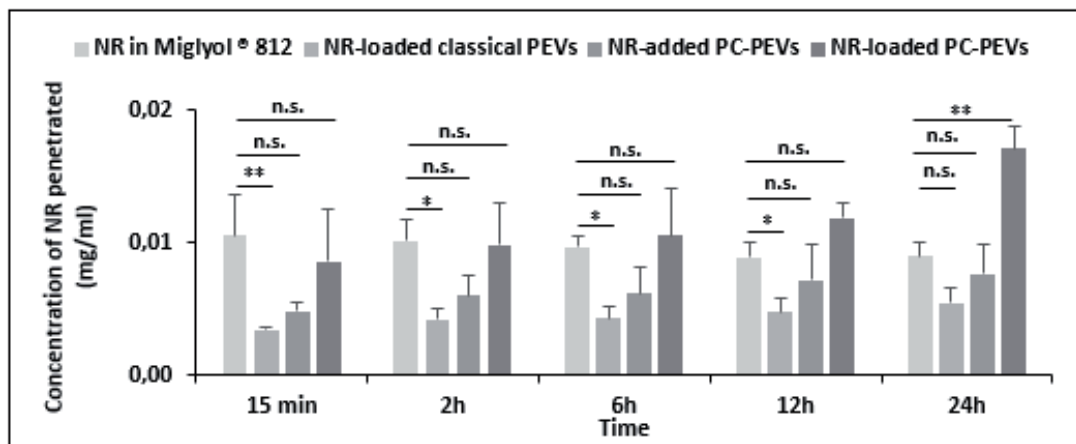


Figure 1: Quantitative dermal penetration of NR from classical PEVs and PC-PEVs (\*  $p < .05$ , \*\*  $p < .01$ , n.s. = non-significant)

## The influence of amorphous stabilization on dermal penetration

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Micellar formulations represent the current standard in dermal care, enhancing both the cleansing efficacy of detergents and the dermal uptake of active ingredients (AI) [1]. With regard to dermal drug delivery, recent studies have demonstrated increased penetration of amorphously stabilized AI compared to micellar and nanosized formulations [2]. This study compares a commercial micellar formulation and amorphously stabilized AI on mesoporous silica, using an ex vivo porcine ear model to determine which approach offers superior biological efficacy.

Syloid® XDP 3050 silica particles (Grace GmbH & Co. KG, Worms, Germany) were loaded with a 2.5 mg/mL solution of curcumin in ethanol using the solvent evaporation method to produce smartPearls®. The amorphous state of curcumin was verified using X-ray diffractometry. An amount of smartPearls® containing 1.5 mg of curcumin was applied to 4 cm<sup>2</sup> of skin with 30 µL of medium-chain triglycerides (MCT, Miglyol® 812) and an equivalent amount of curcumin from the leading commercial formulation was applied and incubated for 2 h at 32 ± 1 °C. After removal of the drug carriers, treated skin areas were excised and sectioned using a cryomicrotome. The obtained skin sections were then analyzed using epifluorescence microscopy (Fig. 1) in combination with digital image analysis.

Curcumin was stabilized in an amorphous state on silica particles, as evidenced by the absence of crystalline reflexes in the X-ray diffractogram. The stratum corneum thickness was increased in the area treated with the MCT-based formulation compared to the micellar-treated area, indicating an occlusive effect. The amount of penetrated curcumin and the penetration depth were both increased compared to the micellar formulation, displaying enhanced dermal penetration of amorphously stabilized curcumin.

The results of the study demonstrate the straightforward loading of lipophilic AI on silica particles in an amorphous state via solvent evaporation, without the need for additional excipients. The observed dermal penetration corroborates previous findings and highlights the enhanced delivery performance of amorphously stabilized actives. Still, additional research is essential to further develop the amorphous stabilization of active ingredients as a viable dermal drug delivery solution.



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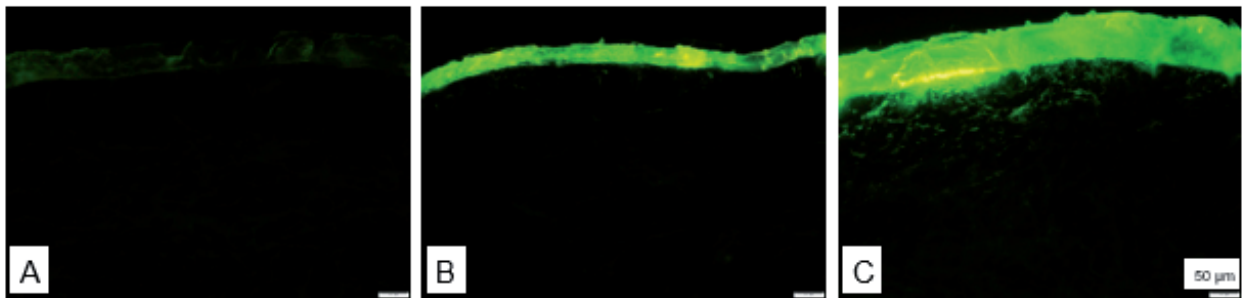


Figure 1: Epifluorescence microscopy images of untreated skin (A), skin treated with a micellar curcumin formulation (B) and skin treated with amorphously stabilized curcumin on mesoporous silica (C); 200-fold magnification.

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# Antimicrobial effects against bacteria, yeast and dermatophytes as well as protease-inhibitory properties of sodium bituminosulfonate, light (SBS)

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Chronic wounds, like venous, pressure and diabetic ulcers, have become a rising charge to society as an increasing number of patients suffer from wounds that fail to heal. This impaired wound healing derives from an imbalance between degradation and remodeling. It was shown that exudates from non-healing wounds contain elevated levels of proteases leading to considerable reduced amounts of growth factors. Chronic wounds are further characterized by an alkaline shift in the wound environment. Bacteria thrive in this protein-rich and alkaline environment. Hence, chronic wounds are often colonized by different kinds of microorganisms, the most prominent being *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The presence of bacteria induces the immigration of monocytes, macrophages and leukocytes, whose inflammatory response exaggerates the tissue damaging processes. Hence, it was postulated that in order to improve the opportunity for wound healing, it is necessary to create conditions that are unfavorable to micro-organisms and favorable for the host repair mechanisms. An additional effect on abundant protease activity in non-healing wounds might be beneficial for using specific wound treatments.

The purpose of the presented comprehensive in vitro approach was to evaluate the antimicrobial activity of sodium bituminosulfonate, light (SBS) against a broad range of clinically relevant microorganisms (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans*, *Candida parapsilosis*, *Trichophyton interdigitale* und *Trichophyton benhamiae*) using microplate-laser-nephelometry (MLN) in accordance with NCCLS M27-A2 and DIN EN 27027. Such in vitro testing allows direct comparison of the effects of the agents on the micro-organisms, is simple, rapid, reproducible, inexpensive, and enables handling of a range of sample quantities. Additionally, the pH effect on the antimicrobial activity was investigated using *Staphylococcus aureus* as test organism. Furthermore, the reduction of the activity of the proteases elastase and collagenase by SBS was investigated.

SBS was able to inhibit the growth of all microorganisms tested. The antimicrobial activity could be classified as a strong antimicrobial effect (log reduction > 3) in all cases according to JISL1902. In addition, SBS showed a bactericidal and fungicidal effect against the bacteria *S. aureus* and *K. pneumoniae* and the microconidia of the dermatophytes *T. interdigitale* and *T. benhamiae*. The IC50 values of the microorganisms against SBS were determined using MLN: *S. aureus* (0.004%) > *T.*



benhamiae (0.1%) > T. interdigitale (0.28%) > K. pneumonia (1.34%) > C. albicans (5.01%) > C. parapsilosis (6.65%). Furthermore, it was found that antibacterial efficacy was significantly enhanced at pH 8 (0.003%) and pH 9 (0.002%) against S. aureus compared to the standard test condition of pH 7. Subsequently, it was shown that SBS is able to significantly reduce collagenase activity in vitro. It was further found that SBS demonstrates a significant capacity to decrease elastase activity in the test.

Sodium bituminosulfonate (SBS) is used in different salves and ointments and exhibits distinct antimicrobial properties. Moreover, it was shown to reduce abundant protease activity in vitro. It could therefore be a potential agent for the therapy of infected and critically colonized chronic wounds.



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# Comparative antibacterial dynamics of sulfonated shale oil derivatives and fusidic acid against Gram-positive pathogens

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The emergence of antimicrobial resistance (AMR) presents a significant global challenge for healthcare systems, particularly due to increasingly limited treatment options, renewing interest in older agents. Ammonium bituminosulfonate (ABS) and sodium bituminosulfonate, light (SBS) show in vitro activity against Gram-positive bacteria like *Staphylococcus aureus*.

This study investigates the antibacterial effects of ABS and SBS compared to fusidic acid (FA) against *Staphylococcus aureus* and *Streptococcus pyogenes*. Test organisms were cultured, and inocula were prepared in tryptic soy broth (Oxoid, U.K). Serial dilutions of ABS (20–1250 µg/mL), SBS (8–500 µg/mL), and FA (1.6–100 µg/mL) were prepared in medium. Concentrations were chosen to detect early antibacterial effects within 4 hours. Bacteria were incubated with the test dilutions at 37 °C for 0.5, 1, 2, 4, 8, and 24 hours. Bacterial viability was quantified using the BacTiter-Glo™ Microbial Cell Viability Assay (Promega), based on ATP luminometry. Viability [%] was determined relative to untreated controls. Dose-response curves were generated using logistic-fit regression (OriginLab Origin 2025) to calculate IC50 and IC90 values.

ABS and SBS showed faster and stronger antibacterial effects against *Streptococcus pyogenes*, with early IC50 reductions. ABS had a delayed but increasing effect on *Staphylococcus aureus*. SBS showed similar patterns but with slightly faster onset. In contrast, fusidic acid (FA) was more effective against *S. aureus*, with rapid IC50 and IC90 reductions, but showed delayed, weaker activity against *S. pyogenes*. Within 24 hours all three substances showed a clear antibacterial activity against *S. aureus* and *S. pyogenes*. Nonetheless, these results highlight distinct, organism-specific activity profiles important for targeted antimicrobial therapy.

