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“Enhancing Deposition Efficiency: The Impact of Glycolipids on Active Ingredients”

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1. Introduction

In today's beauty and personal care landscape, consumers are not only looking for effective cleansing solutions but are also concerned about how these products influence the condition of their skin and hair. The inclusion of active ingredients that promote hydration, nourishment, and skin/scalp barrier support is therefore becoming essential in cleansing applications. As a result, there is a growing demand for water-based formulations that combine cleansing with additional benefits like anti-aging, hydration, repairing and overall skin and scalp/hair health. Moreover, mildness has emerged as a crucial factor in product selection. Gentle formulations that cleanse without stripping the skin of its natural moisture or disrupting the skin barrier are highly sought after, as consumers prioritize comfort and skin integrity. By focusing on the dual role of cleansing and caring, manufacturers can create products that meet the evolving expectations of consumers who desire effective yet gentle cleansing solutions that enhance their overall skin condition.

Of the many different ingredients on the market fitting into this trend, Ceramides became one of the most popular active ingredients in the personal care market as their efficacy was demonstrated in many different studies.^[1-5] Ceramides are the major lipid constituent of lamellar layers present in the intercellular spaces of the stratum corneum. These lamellar structures in combination with the corneocytes build the skin barrier, an essential structure to prevent water loss and to keep the skin functional. Furthermore, Ceramides are also found in hair cuticle. They form part of the lipid matrix that holds cuticle cells together, enhancing the protective barrier. They are essential for maintaining the structural integrity of the hair shaft. The naturally occurring amount of ceramides is reduced in many different skin conditions due to extrinsic and intrinsic influences as well as in the hair tips, therefore replenishing ceramides to skin and hair is a very effective way to improve overall condition of skin and hair.^[6,7]

The inherent tendency to crystallize and the hydrophobic nature of Ceramides poses significant technical challenges to incorporate them into water-based systems.^[8,9] This crystallization does not only negatively effects formulation stability but also raises concerns about the bioavailability of these essential lipids in rinse-off products. Many consumers intuitively question

the rationale behind using Ceramides in rinse-off-formulations, as the beneficial ingredients are potentially simply flushed down the drain, negating their potential benefits.

However, recent advancements in formulation science, particularly the use of nature-identical Glycolipids derived by fermentation, provide a compelling solution to this dilemma.^[10,11] Glycolipids serve as effective solubilizers and deposition agents, enhancing the ability of Ceramides to adhere to the skin and hair even in rinse-off applications.^[12-14] This means that, rather than being predominantly lost during the washing process, Ceramides can be more effectively deposited onto the skin's surface, where they can exert their moisturizing and protective benefits. By harnessing the unique properties of Glycolipids, formulators can create innovative products that not only address the technical challenges of incorporating Ceramides but also align with consumer expectations.^[15] This approach does not only maximize the efficacy of Ceramides but also reinforces the value of using such ingredients in rinse-off products, ultimately leading to a more satisfying and effective consumer experience. In a market increasingly focused on sustainability and efficacy, the integration of Glycolipids as supportive agents for formulating Ceramides represents a promising frontier in cosmetic science.

2. Materials and Methods

Preparation of the Blend

Ceramide was added to a solution of Glycolipids in water and heated at 50°C for 10 minutes. After cooling to room temperature, a clear yellowish solution was received.

In the following studies, either the concentrated combination of Glycolipids and Ceramides was used, or when described a diluted form was used. In the latter case 5% of the blend was diluted in water and adjusted to a pH value of 7.

Microscopic Analysis

For polarized light microscopic analysis, the Olympus BX-53 was used as a device, that has two components needed for the measurement: a polarizer and an analyzer. The polarizer produces linearly polarized light to illuminate the sample, while the analyzer, positioned at a 90° angle to the polarizer, restricts the passage of light to only refracted light with a specific polarization. This setup creates a "dark position," where no light passes to the camera or eyepieces unless it has experienced a change in polarization after passing through the sample. This ensures that only birefringent light, altered by the sample, is visible. If crystals are present in the sample they will appear as white objects in microscopic images.

In this study, the concentrated samples have been used, showing that the Ceramides can be successfully solubilized in Glycolipids. As clearly shown in Figure 1, no crystal formation could be observed in the blend of Glycolipids and Ceramides.



Figure 1: Microscopic data of the Glycolipids and Ceramides blend showing no crystal formation.

Polarized Filter Analysis

To further confirm the findings of the microscopic results, the concentrated solution of Glycolipids and Ceramides was additionally examined through a polarized filter. Polarized filters work by only allowing light waves vibrating in a specific direction to pass through. Unpolarized light vibrates in all directions perpendicular to its travel path, but a polarized filter blocks all vibrations except for those aligned with its polarization axis.

Based on these properties polarized filters are useful for visualizing crystals in solutions because crystals are birefringent—they split incoming polarized light into two rays that travel at different speeds and exit vibrating in different directions. When polarized light passes through these crystals (between two crossed polarizers), the differences in light speed and direction create interference patterns or changes in brightness and color. This makes crystals stand out clearly against the background, even when they are tiny or nearly invisible in normal light.

As can be seen in Figure 2, no crystals could be observed in the concentrated blend of Glycolipids and Ceramides further confirming they were successfully solubilized in Glycolipids.



Figure 2: Image of Glycolipids-Ceramide combination through polarized filter. No crystal formation can be observed.

Dynamic Light Scattering

Dynamic Light Scattering (DLS) is used to detect crystals (or incipient crystallization) in solutions by measuring the particle size distribution based on particle diffusion. In a solution, aggregates, including small crystals, undergo Brownian motion. DLS analyzes the fluctuations in scattered light intensity caused by this motion to determine particle size. The appearance of larger, slower-moving particles may indicate aggregation or, in this case, crystal formation, making DLS a sensitive, non-invasive method for monitoring crystallization processes.

DLS Measurements were carried out using a NanoLab 3D device from LS Instruments AG. The instrument is equipped with a 120 mW laser operating at a wavelength of 638 nm and measured at a measurement angle of 90° to the incident beam. Samples were measured unfiltered, in sealed 12 mm square disposable polystyrene cuvettes. For each sample, 3 replicate measurements were performed at 23°C for 45 seconds.

For this study the diluted form of the Glycolipids-Ceramide blend in water was used comparing it to a dilution of the single components used for the Glycolipids-Ceramide blend. These diluted samples should further demonstrate the benefits of the Glycolipids and Ceramides blend in water-based personal care formulations as they are not resulting in any crystal formation. Each sample is a 5% a.m. mixture using the same ratio of Glycolipids and Ceramides in water. The first sample was prepared using the pre-mixed blend (Figure 3; left), the second sample contains the individual ingredients added one at a time without using the pre-mixed blend (Figure

3; right). Both samples were dissolved in water, adjusted to pH 7, and prepared using a cold process.

A comparison of the two samples prior to measurement revealed that only the premixed sample yielded a clear solution. The presence of crystals in the other sample was evident. Consequently, the measurement of the DLS was conducted exclusively on the clear sample.

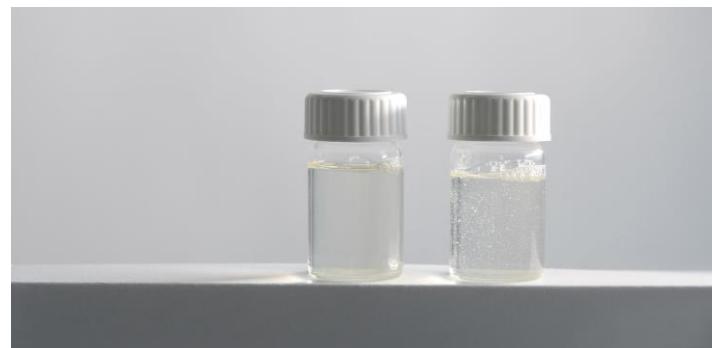


Figure 3: Visual comparison of the Glycolipid-Ceramide blend and its single raw materials diluted in water and adjusted to a pH value of 7.

Glycolipids are known to form micelles in water. In Figure 4 the DLS autocorrelation function of pure Glycolipid at pH 7 in water shows only a rapid decrease, indicating small, monodisperse micelles with a radius of 1.3 nm.

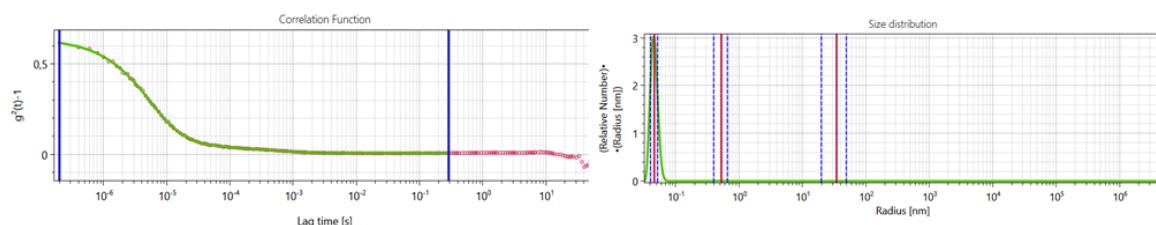


Figure 4: DLS autocorrelation function (left) and particle size distribution (right) of Glycolipid at pH 7 in water.

The sample prepared from the premixed blend was measured using DLS as well. Figure 5 also exhibited a monomodal decay, suggesting the presence of a single size distribution. This finding indicates that no crystals but only small surfactant micelles were formed in the sample.

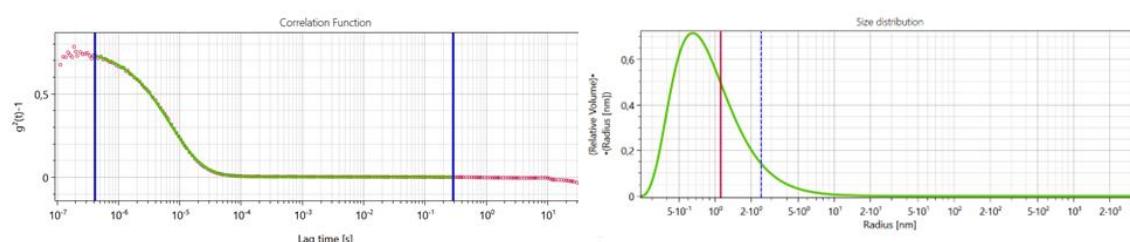


Figure 5: DLS autocorrelation function (left) and particle size distribution (right) of the pre-mixed blend of Glycolipids and Ceramides.

Formulation Compatibility

To further demonstrate the compatibility of the Glycolipid-Ceramide blend in water-based formulations a final body wash formulation was prepared. The formulation, including additional surfactants and thickening agents is clear and does not show any crystals using the same methods as described above (figure 6).



Figure 6: Body Wash Formulation including the pre-mixed combination of Glycolipids (and) Ceramides.

Application Studies

To further elaborate the benefits of Glycolipids and Ceramides, deposition studies have been performed on skin and hair respectively to create valid and trustful data that shows it is worthwhile using them in rinse-off applications even though they have a short contact time with the surfaces.

Deposition On Skin

To ensure the reproducibility of the study all panelists ($n = 10-12$) undertook the same pre-treatment in the climate room. To ensure that all skin types were represented different genders and ages ($m = 3$, $f = 8$; ages between 25-60) were included.

To evaluate the deposition and effectiveness of a test formulation on the skin, a systematic method was employed. The process began with a pre-wash of the forearms using a solution of Sodium Lauryl Ether Sulfate (SLES) and Cocamidopropyl Betaine (CAPB) in a 9:3 ratio lasting 10 seconds, followed by a thorough rinse with water for another 10 seconds to remove any residual surfactant. After the initial cleansing, the panelists' forearms were conditioned in an environment with 50% relative humidity at a temperature of 22°C for 15 minutes. This conditioning period is crucial for simulating skin conditions prior to the application of the test formulation.

Once conditioned, specific areas measuring 6.25 cm² were marked on the forearms for the application of the test formulation. These areas were clearly labeled to ensure accurate identification. Before applying the formulation, baseline samples were taken using sampling strips, collecting three samples from the marked areas. This establishes a reference point for evaluating the impact of the formulation. For this study the assumption was made that the baseline sampling results applied to the whole marked area.

The test formulation was applied to the marked areas next to the baseline samples (still within the marked areas) as the baseline area is expected to be slightly damaged which might enhance deposition. The test formulation was applied using an Eppendorf pipette, delivering 20

μL of the formulation three times, for a total of 60 μL . After each application, the formulation was gently massaged in five circular motions using gloves, followed by 20 final circle movements to ensure even distribution and penetration of the formulation into the skin.

Following the application, the formulation remained on the skin for one minute. After this waiting period, the treated areas were rinsed carefully with 60 mL of water, followed by careful drying with a towel. After this rinse, the forearms were allowed to rest for an additional 10 minutes before samples were taken from the marked areas using stripes three times and the same way it was done for the baseline measurements. This comprehensive approach provides valuable insights into the deposition characteristics and overall effectiveness of the test formulation on the skin.

The amount of Glycolipids and Ceramides on the skin before and after the rinse-off application were measured using a suitable high performance liquid chromatography (HPLC) method. To ensure a high sensitivity for the targeted analysis, a liquid chromatography–mass spectrometry (LC-MS) system was used.

Before the measurement, the lipids were extracted from the test strips by covering the samples with a defined volume of organic solvent and subjecting them to ultrasonic treatment. An aliquot of this solution was then analyzed using LC-MS. To quantify the content of the respective lipids, external calibration functions were established using qualified reference materials.

3. Results

As Ceramides are part of the stratum corneum it is not surprising that they can be detected in average concentrations of 0.41 $\mu\text{g}/\text{mL}$ on the skin (Figure 7, Before). After application of the Glycolipid-Ceramide blend using a rinse-off application, the amount of Ceramides detected on the skin increased to a value of 1.32 $\mu\text{g}/\text{mL}$, which clearly indicates a deposition of the Ceramides. The total Ceramide content increased by +221% (Figure 7, After).

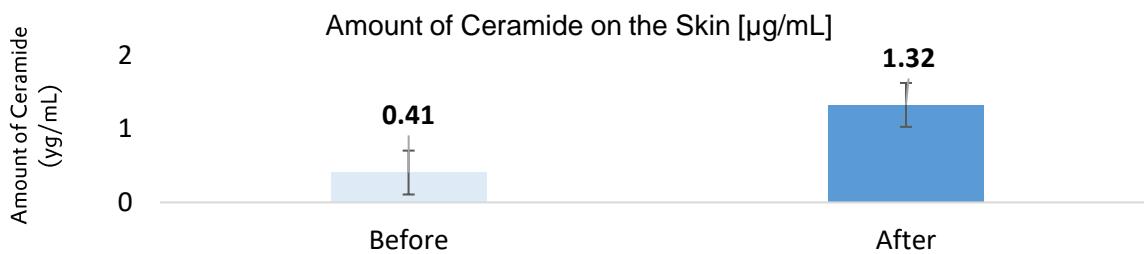


Figure 7: Amount of Ceramide on the skin before and after application.

Deposition On Hair

To ensure its consistency $n = 10$ hair tresses were used for the study. In a first step, each hair tress was pre-washed two times using a 12% SLES solution: the hair tresses were massaged with a defined amount of shampoo for one minute, rinsed for 30 seconds, massaged with the same shampoo again for one minute, and then rinsed for one minute. After this pre-wash, the hair tresses were left to dry overnight in a climate-controlled room. Once dry, the hair tress

was split lengthwise to separate the same tress and use it for the most comparable results before and after the treatment.

To analyse the amount of Glycolipids and Ceramides on the hair tresses before treatment no additional steps were performed. The hair tress was cut into short pieces and weighed into a small vial.

The hair tresses used for the actual deposition study were with water and per 2 g of Hair tress, 1.5 g of a shampoo including 5% of the Glycolipid-Ceramide blend was applied on a watch glass and slightly massaged in for one minute, followed by rinsing with a defined amount of water. This step was repeated for a second time and the hair tresses left to dry overnight.

The after-treatment hair tresses were each cut into short pieces and weighed into a small vial.

Similar to the skin deposition study, a suitable high performance liquid chromatography (HPLC) method was developed to determine the content of Ceramides and Glycolipids in the respective samples. To ensure a high sensitivity for the targeted analysis, a liquid chromatography–mass spectrometry (LC-MS) system was used.

Before measurement, the lipids were extracted from the hair tresses by covering the samples with a defined volume of organic solvent and subjecting them to ultrasonic treatment. An aliquot of this solution was then analyzed using LC-MS. To quantify the content of the respective lipids, external calibration functions were established using qualified reference materials.

This study on hair tresses is currently ongoing to showcase that a deposition of Ceramides is also enabled on hair surface, further supporting that Ceramides can be included in hair care rinse-off formulations. No final results can be shared yet but first HPLC data show detectable amounts of Ceramides and Glycolipids on hair surface comparing it to non-treated hair tressed.

Make Up Removal

To translate these results into final applications we have tested the performance of the Glycolipid-Ceramide blend using a make-up removal test, to mimic final consumer application.

The cleansing efficacy was evaluated by removing make-up from PMMA plates using a scrub abrasion and washability device. As a starting point, the color value of the PMMA plate was measured at three predetermined positions using the appropriate positioning devices. Next, a defined amount of makeup was applied using a bar applicator. After allowing the make-up to dry for one hour, the color value of the coated PMMA plate was measured again at the same three positions. The plate was secured in place, and 2.5 g of the respective test solution was applied to a cotton pad. This cotton pad was then attached to the sponge of the sliding carriage and attached to the device. The cycles were set such that one cycle consists of one forward and backward movement, operating at a speed of 10 cycles per minute, with each cycle covering 160 mm. After another hour of drying, the color value on the plate was measured at the three designated positions once again. The make-up removal properties were determined using the ΔE values (Δ empty plate/coated plate and Δ coated plate/cleaned plate). For this study 3% of the concentrated Glycolipids-Ceramide blend was diluted in water and adjusted to a pH value of 7.

The results show a successful removal of 94% of the initially applied make-up using the Glycolipids-Ceramide blend. This indicates that the blend does not only bring benefits to the skin by the deposition of Ceramides, but also has a very gentle yet effective cleansing given by the Glycolipids.

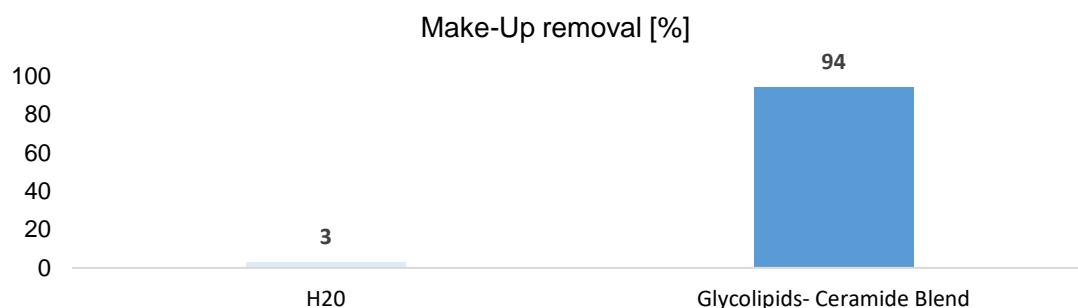


Figure 8: Make up removal efficiency of the Glycolipids-Ceramide Blend.

4. Discussion

These studies successfully demonstrate the effective integration of Ceramides solubilized by Glycolipids into water-based formulations, solving a yet unmet formulation need and paving the way for a new generation of innovative solutions in the personal care market: Clear water-based Ceramide containing products combined with the well-known Ceramide-benefits. The combination of these ingredients did not only result in clear and stable systems but also showcase their potential in rinse-off applications. Analytical techniques, including polarized light microscopy and dynamic light scattering confirmed the absence of crystal formation, indicating successful solubilization in concentrated but also diluted form, a water-based end-customer formulation. Furthermore, deposition studies on both skin and hair highlight the enhanced retention of active ingredients, attributed to the unique properties of Glycolipids. These studies clearly show that a deposition of ceramides on the skin is given after a rinse off formulation following a standardized protocol by combining two key technologies.

First results on the deposition of ceramides on hair after a rinse off application already showed detectable differences indicating a successful deposition. A bigger study is currently running to perform statistically significant results but also to be able to include different ethnicities.

To our knowledge, this is the first instance where a significant deposition of ceramides from a rinse-off formulation utilizing only glycolipid technology has been reported. While there are similar products available that employ more complex technologies, this straightforward yet effective solution paves the way for new formulations that are accessible to all customers and consumers, resulting in more efficient and impactful outcomes.

Moreover, these findings highlight the ongoing importance of this area of key technologies, revealing that several mechanisms remain unknown and not fully understood. This study enhances our understanding of ceramide deposition and opens avenues for further research, supporting the benefits of this deposition for both hair and skin applications.

5. Conclusion

Ceramides, recognized for their role in maintaining the skin and hair barrier, have emerged as a key ingredient in this trend. However, their incorporation into rinse-off formulations has historically posed challenges due to their hydrophobic nature and tendency to crystallize. Recent advancements in formulation science, particularly the use of Glycolipids, present a promising solution by enhancing the solubilization and deposition of Ceramides. This innovative approach not only addresses technical challenges but also aligns with consumer expectations for effective, gentle cleansing solutions.

The findings from this study demonstrate that the combination of Glycolipids and Ceramides can successfully yield stable, clear water-based formulations that effectively deliver the benefits of ceramides to both skin and hair. The significant increase in ceramide deposition observed in both skin and hair after rinse-off applications validates the efficacy of this approach. As the personal care market continues to prioritize sustainability and efficacy, the integration of glycolipids as supportive agents for ceramide formulation represents a significant advancement in cosmetic science.

This research opens avenues for further exploration into the mechanisms of ceramide deposition, paving the way for the development of more innovative and effective personal care products that meet the evolving needs of consumers.

6. Literature Data

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