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HENRY MASO KEYNOTE AWARD LECTURE

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GREEN CHEMISTRY: THE MISSING ELEMENT OF MATERIALS DESIGN

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Warner Babcock Institute for Green Chemistry, LLC

Imagine a world where all segments of society demanded environmentally benign products. Imagine if all consumers, all retailers and all manufacturers insisted on buying and selling only non-toxic materials, environmentally benign materials. The unfortunate reality is that, even if this situation were to occur, our knowledge of materials science and chemistry would allow us to provide only a small fraction of the products and materials that our economy is based upon. The way we learn and teach chemistry and materials science is for the most part void of any information regarding mechanisms of toxicity and environmental harm. Green Chemistry is a philosophy that seeks to reduce or eliminate the use of hazardous materials at the design stage of a materials process. It has been demonstrated that materials and products CAN be designed with negligible impact on human health and the environment while still being economically competitive and successful in the marketplace. This presentation will describe the history and background of Green Chemistry and discuss the opportunities (environmental and economic) for future products based on the principles of green chemistry.

DEVELOPMENT OF SENSORY TEST METHODOLOGY FOR THE IDENTIFICATION OF SUSTAINABLE POLYMERS FOR HAIR STYLING APPLICATIONS

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Introduction: There is an increasing interest to develop and use sustainable polymers in place of synthetic styling polymers for hair care applications. Styling products provide temporary styling benefits to retain shape and style in hair, and are applied to dampened hair after shampooing and/or conditioning, or to dry hair. These products should be readily removed by water or shampoo. Synthetic polymers offer advantages to the formulator, such as water and/or ethanol solubility, good hold and film formation. However, the disadvantages of synthetic polymers from a green chemistry perspective most notably include lack of sustainability, renewability, and biodegradability among other environmental concerns.[1] Naturally derived styling polymer alternatives, such as starches, polysaccharides and gums, are well known to formulators in the industry. The limitations of these current options are also well known and may include formulations issues such as lack of solubility, high viscosity or other undesirable properties.

The development of a sensory testing method to identify and characterize the formulation benefits of naturally-derived polymers has been undertaken. Using this method, seven common synthetic styling polymers and more than two dozen naturally-derived polymer alternatives have been characterized to date. A combination of swatch testing, sensory evaluations and polymer characterization is being used. This allows for direct comparisons of synthetic styling polymers to natural polymers; the example below compares PVP to different starch (potato and corn) derivatives. The resulting data has led to the successful replacement of synthetic polymers in a number of newly developed hair styling products.

Methodology: Each polymer was characterized in the same manner, from an aqueous gel formulation. The gel base with no polymer added was used as the control. Sensory ratings are normalized such that a rating of 5 indicates the most positive results for each attribute. Hair swatches were cut to ¼" and 0.30 g of product was applied to swatches that had been shampoo washed and air dried.

High humidity curl retention tests were conducted from tresses rolled onto a curler, which were allowed to air dry for a minimum of one hour. The dried tresses were carefully unwrapped and hung from a clip bar with a ruler. Curl retention measurements were taken in a humidity chamber at 90 °F, 90% RH. Readings were taken at 10 and 60 minutes, and curl retention is calculated as:

$$\% \text{ Curl retention} = (L - L_t)/(L\text{-}L_o) \times 100 \ ^{(2)}$$

Where L is the length of the uncurled tress, L_t is the length of the curled tress at the time of the reading and L_o is the length of the curled tress at the start of the experiment.

Results: From the data shown below (Figure 1), the control gel is rated higher than the polymers for shine, natural feel and flaking, which indicates that it is an appropriate control for these analyses. Addition of film forming polymers to the gel leads to increased hold on hair swatches. The modified corn starch polymer was rated highest for hold, and left the hair swatch feeling natural, not coated. Very high flaking is the main drawback of the corn starch polymer, while the potato starch had acceptable ratings for shine, flaking and feel on hair.

Also included are high humidity curl retention results (Figure 2), which are a measure of how well the naturally derived polymer film will hold a curl in real-life conditions. The PVP film maintains the greatest amount of curl with high humidity and temperature, while the control gel retains the least amount after 60 minutes. Both modified starch polymers performed similarly and maintained approximately 50% of the curl length for 60 minutes.

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[2] Zviak, C.; *The Science of Hair Care*; Zviak, C., ed.; Marcel Dekker; p. 169; *1986.*

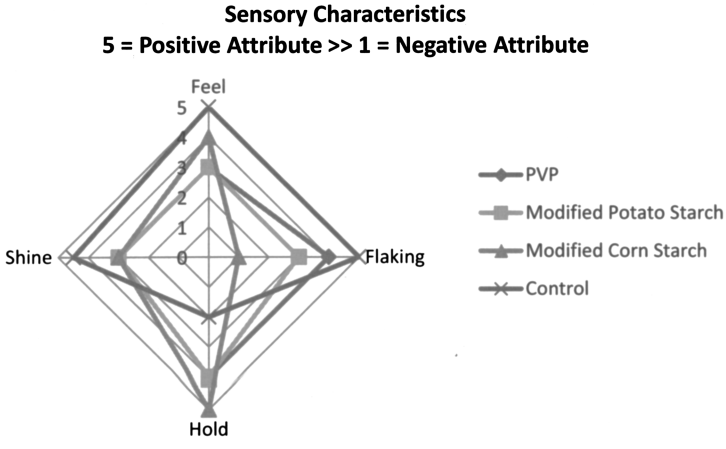

Figure 1: Sensory data comparing modified corn and potato starch and PVP gels (5% active) to a control gel with no polymer added. Results of shine, feel, flaking and hold assessments are shown. (Scale is calibrated so that 5 is the most positive rating for each attribute.)

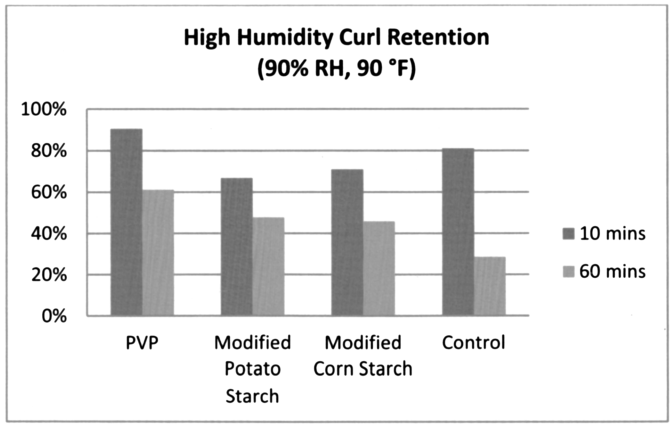

Figure 2: High humidity curl retention results comparing PVP, modified corn and potato starch gels (5% active) to a control gel with no polymer added.

Conclusion: Sensory testing can be successfully used as a formulation tool early in the product development process to assist in the replacement of synthetic ingredients with natural or naturally-derived, sustainable alternatives. A comprehensive study has been completed in which a wide variety of polymers were characterized by sensory methods at an earlier point in the product development process. From this data, formulators are able to anticipate the positive and negative aspects of a naturally derived polymer before they begin using it in final, consumer acceptable formulations.

GREENING THE SUPPLY CHAIN TO DEVELOP MORE SUSTAINABLE FORMULATIONS

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Objective:

Formulator's rely heavily on their supply chain in order to develop more sustainable products while meeting the needs of their customers. The ACS GCI Formulator's Roundtable was developed to be the driving force to use green chemistry in creating innovative products that are environmentally sustainable throughout their product life cycle and safer to make and use. The Roundtable, comprised of companies significantly involved in the formulation of soap, detergents and cleaning preparations and/or perfumes, cosmetics, and other toilet preparations, acknowledges its position to generate an aggregate demand for greener alternatives to currently used raw materials. The components of existing formulated products are considered safe and effective; however, it is the intention of the Roundtable to foster the development of innovative greener components to enhance the overall sustainable profile of formulated products.

Methodology:

To initiate progress towards informing and influencing suppliers and academia to develop greener alternatives, the Roundtable believed it was imperative to define the top areas for opportunities for greener alternatives as identified from a formulator's perspective. It was not sufficient to simply list the areas; the Roundtable felt it was critical to define the preferred characteristics of each. Focusing largely on commodity types of raw materials and avoiding proprietary ingredients, the list was developed with input and review from all member companies. The opportunities are common to the industry and do not represent one particular company's interests. The final list was the consensus view of the Roundtable members and reflects data available at the time of the project.

Results:

Preferred characteristics were identified for the following opportunities as defined by the ACS GCI Formulator's Roundtable:

- Greener "Antimicrobials"
- Greener Solvents
- Greener Small Amines, MEA, DEA, TEA
- Greener Chelants
- Greener Boron Alternatives
- Greener Fragrance Raw Materials
- Greener Corrosion Inhibitors
- Greener Alkanolamide Replacements
- Greener Surfactants
- Greener UV Filters

Conclusions:

This list of opportunities for greener alternatives from a formulator's perspective is not intended to be exclusionary, but rather provide an initiation point. The ACS GCI Formulator's Roundtable recognizes this as an opportunity for collaboration where appropriate, for funding where feasible, and for global communication as needed to engage the broader audience in this effort to bring greener alternatives into the marketplace. It is the hope of the Roundtable that this list of top formulation opportunities initiates dialogue, research, and development of renewable and less hazardous alternatives.

THE ANSI/NSF/GCI 355 STANDARD: A TOOL FOR GREENER FORMULATIONS

Robert Peoples*, Ph.D. and Shefali Algoo
American Chemical Society
*Please note Dr. David Wylie will make the presentation on behalf of Dr. Peoples.

OBJECTIVE

The foundation of our modern chemical infrastructure is rooted in deciphering the rules of chemistry in the late 19th and early 20th centuries. Coupled with a cheap and plentiful supply of both energy and chemical building blocks derived from petroleum, the modern chemical enterprise blossomed.

In 1962 with the publication of "Silent Spring," by Rachael Carson, a real awareness of the impact of mankind on our environment entered our collective consciousness. For the first time we began to understand the implications of the modern chemical industry.

The purpose of the research and creation of the NSF/GCI/ANSI Standard is to provide business to business communication with greater clarity and information to design benign chemicals, one of the biggest hurdles for companies up and down the supply chain. Coupled with growing awareness of the demands of an expanding population, rising standards of living and the scale of resources and energy required to meet those demands, the new rules of sustainable design are inextricably coupled with newer standards and regulatory frameworks.

METHODOLOGY

The American Chemical Society Green Chemistry Institute® (ACS/GCI) partnered with over forty stakeholders to develop a standardized B-2-B tool which helps chemists evaluate the impact of chemicals, processes and design of new molecules in an environmental health safety framework. It is expected that this Standard will help to provide vital information on other approaches that have evolved to evaluate the relative sustainability of a chemical product including: hazard analysis, risk assessment, eco-efficiency analysis, carbon footprint, and life-cycle assessment.

This Standard is the first to incorporate both information on the chemical hazards and also the manufacturing process considerations in a unified format. It assists chemical manufacturers and formulators who need to know the identity and hazard profiles of the chemical ingredients used in the products and the impacts associated with the manufacturing process.

Thanks to a two-and-a-half-year cooperative effort between NSF International and ASC/GCI there is now an official ANSI standard.

RESULTS

NSF International, along with the ACS/GCI, has released an American National Standard to provide the chemical enterprise with a voluntary and standardized way to define and report on a chemical product's hazard profile and its associated manufacturing process impacts. The standard provides reporting guidance on product identification, chemical characteristics such as human health effects, ecological effects, physical safety properties, and gate to gate process impacts including recycled/reused content, water use, energy use, bio-based content, process safety, and innovative technologies. The NSF/GCI 355 *Standard for Greener Chemicals and Processes Information* provides a clear, consistent and transparent way to communicate this information from business to business throughout the supply chain. The information generated through the standard will assist customers in evaluating the relative sustainability of a chemical product and process over its life cycle.

CONCLUSION

The Standard will play a central role in the transformation of chemistry from a petroleum-based enterprise, to one driven by the 12 principles of green chemistry and engineering. Such a shift will be of interest to the cosmetics industry as new materials and sources become manifest. This talk will review global perspectives driving these changes, examples of the successful implementation of green chemistry and engineering, and highlight the role of the Institute's programs in aiding this transformation.

HPTLC- SEPARATION, QUANTIFICATION AND IDENTIFICATION OF LIPIDS IN SKIN AND COSMETICS BY USING AUTOMATED MULTIPLE DEVELOPMENT (AMD) AND TLC- MS- INTERFACE

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Anhalt University of Applied Sciences

The aim was to develop an optimized chromatographic separation method of various lipid substances of stratum corneum and of lipids in cosmetics by using AMD. AMD is a powerful tool in HPTLC for high separation performance and allows qualification and quantification over a wide range of polarity.
The combination of HPTLC with TLC-MS-Interface allows a fast identification of lipids directly from the thin layer plate by using mass spectrometry.
A successful separation of skin lipids could be done. The gradient consisting of 4 solvents (chloroform, hexane, methanol and toluene) could separate the most important stratum corneum lipids in just 8 steps within 1 h 35 min. The developed HPTLC method allows the determination of skin lipids in real patient samples. The quantification working ranges varied between 8,65 ng to 2730 ng (depending on lipid). Calibration functions showed correlation coefficients from 0.97 to 0.99.
A clear identification of substances was achieved by using direct coupling between TLC-plate and mass spectrometry. TLC-MS Interface is very quick and easy to handle.

LIPIDIC HOMEOSTASIS IS ESSENTIAL TO MAINTAIN SKIN BARRIER STRUCTURE AND FUNCTION THROUGH AGING AND ENVIROMENTAL INSULTS

Isabelle Imbert[1], Ph.D., Jean Marie Botto[1], Ph.D., Karine Cucumel[1], Ph.D., Catherine Gondran[1], Ludivine Mur[1], Laurine Bergeron[1], Christelle Plaza[1], Gopi Menon[2], Ph.D., Yolene Guerif-Ferreira[1], Arlette Bergui[1], Gilles Oberto[1], Claude Dal Farra[2], Ph.D. and Nouha Domloge[1], M.D.

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Introduction:

The objective of this work was to study and evaluate new compounds targeting three major biological pathways essential for skin barrier permeability, function and recovery [1]. As cholesterol synthesis is essential to maintain optimal barrier permeability, we evaluated a natural compound (IV09.001) for its capacity to modulate 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase in skin, the rate-limiting enzyme in Cholesterol synthesis [2].
Among others essential pathways involved in the maintenance of skin barrier function and recovery, we investigated the effects of a peptide (IV08.003) on caspase 14, a member of the cysteinyl aspartate-specific proteases, activated during terminal differentiation of the epidermis [3]. Finally, a second natural compound (IV08.008) was also studied for its role in the formation of the cornified envelope by modulating transglutaminases, calcium-dependent enzymes that catalyze the formation of ε-(γ-glutamyl) lysine bonds [4].

Methodology:

Nile red staining was used to observe epidermal lipid content and histochemistry was performed to evaluate expression of HMGCoA reductase, transglutaminases and caspase 14 on human skin biopsies. Electron microscopy was useful to study lamellar bodies content. QPCR was investigated to evaluate mRNA expression of the two major skin antimicrobial peptides, βdefensin 1 (DEFB1) and cathelicidin (LL-37). Finally, H&E staining were performed to observe the morphology and structure of the epidermis after UV insults or tape stripping. Benefits observed on *ex vivo* skin were completed by *in vivo* confocal microscopy investigations.

Results:

In human skin biopsies, topical application of a cream containing 1% of IV09.001 noticeably increased the expression of HMG-CoA reductase after 48h (+78.12%), compared to the placebo-treated samples (Figure 1).
Stimulation of HMGCoA reductase by IV09.001 increased polar epidermal lipids (+41.4% after 48h) as well as lamellar body (LB) content in the upper layers of the *stratum granulosum* and at the *stratum granulosum-stratum corneum* interface after 72h (Figures 2 and 3).

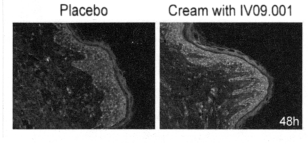

Fig 1: Immunofluorescence staining of HMG-CoA reductase on human skin biopsies treated with a cream containing IV09.001 at 1% for 48h.

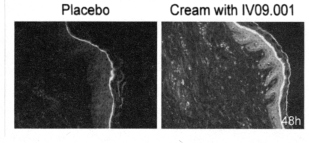

Fig 2: Nile red staining of human skin biopsies treated with a cream containing IV09.001 for 48h.

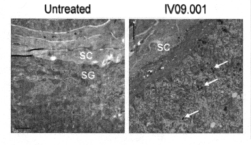

Fig 3: Electron microscopy images of *ex vivo* human skin treated for 72h with IV09.001 at 1%.

Enhanced expression of antimicrobial peptides DEFB1 (+31%) and LL-37 (+23%) was also observed after 48h treatment with the HMGCoA reductase inducteur (Figure 4).

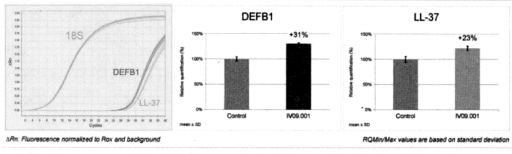

Fig 4: qPCR analysis of DEFB1 and LL-37 mRNAs expression levels in NHK treated with 1% for 48h.

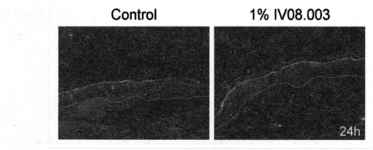

Fig 5: Immunostaining of caspase 14 on human skin biopsies treated with IV08.003 at 1% for 24h.

Experiments conducted with IV08.003 on skin biopsies showed that caspase-14 expression was induced within 24h of treatment at 1% (+224%) as observed on figure 5. In skin biopsies where caspase 14 was pre-induced, we observed a significant reduction of skin damages induced by UVB (-34% of CPD, -39% SBC) compared to untreated biopsies (Figures 6 and 7).

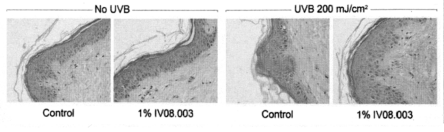

Fig 6: H&E staining on human skin biopsies treated with IV08.003 for 24h and irradiated with UVB at 200mJ/cm^2

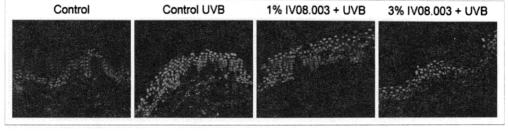

Fig7: Immunostaining of CPDs on human skin biopsies treated with IV08.003 for 24h and irradiated with UVB at 200mJ/cm^2

The effect of compound IV08.008 was finally investigated on transglutaminases. A significant increase of transglutaminases activity was observed after 48h (+47.7%). Effect on Transglutaminase 1 expression as well as the substrate molecules involucrin and loricrin were also evidenced (Figures 8 and 9).

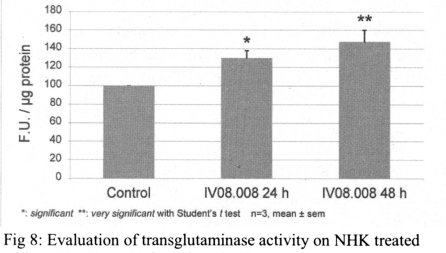

Fig 8: Evaluation of transglutaminase activity on NHK treated with IV08.008 at 1% for 24h and 48h.

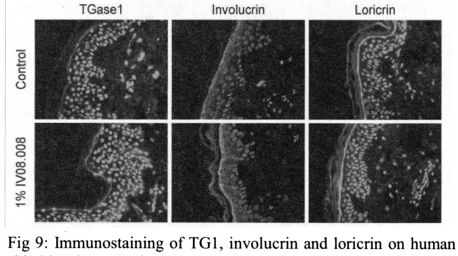

Fig 9: Immunostaining of TG1, involucrin and loricrin on human skin biopsies treated with IV08.008 at 1% for 24h

Increase of transglutaminases activity showed a significant improvement in stratum corneum resistance and moisturization as observed by vivascope study (data not shown).

Conclusion:

These studies highlight the skin benefits of compounds maintaining lipidic homeostasis by modulating HMGCoA reductase, caspase 14, transglutaminases and improving epidermal barrier structure and function as well as resistance to stress and pathogens. These results suggest great promise especially for aged skin that may suffer from decline in lipids synthesis and reduced UV protection and DNA repair capacity.

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SECRETS TO IMPROVING SKIN BARRIER FUNCTION AT THE CELLULAR LEVEL

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Introduction

Optimum skin barrier function is the primary line of defense against environmental stresses such as UV-induced photo-damage, insults from microbial infections and physical breakdown resulting from wound formation. In compromised skin, effective barrier function is critical for mitigating acute and chronic damage to the epidermis and preventing water loss. In the past years, several topical ingredients have been developed to alleviate compromised barrier in an effort to improve overall appearance of the skin. Our research focuses on developing bioactive fermentation extracts **(BFEs)** by proprietary fermentation methodologies that produce a competitive-type response within the microbes during fermentation. BFEs were generated to influence biological markers that are critical for effective barrier function at the *cellular* level.

Our research focuses on two biomarkers critical for optimal barrier functionality, namely hyaluronic acid and Caspase-14. Hyaluronic acid is an integral part of the extracellular matrix that is responsible for maintaining adequate cellular moisturization. Caspase-14 is mainly found in epidermal cells and belongs to a group of proteins that are critical for cellular differentiation and barrier formation.

Methodology

In vitro analysis:

An *in vitro* wound simulation model was developed using MatTek® skin equivalent tissues to mimic compromised skin and evaluate histological changes in biological markers that are critical for barrier function. The tissues were wounded using a 4mm biopsy punch to remove the epidermal layer and then treated with test materials (BFEs). The following samples were evaluated in the wound simulation model:

1. Untreated
2. 2% Dual Ferment

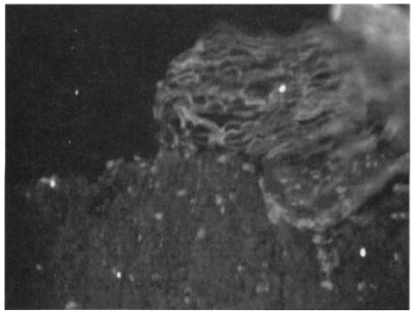
After the treatment process, the tissues were washed with 100µl of PBS, fixed and prepared for histological processing. The tissues were stained using the appropriate immune-fluorescent dyes and evaluated using a confocal fluorescence microscope equipped with a QiClick imaging camera; changes in biomarkers were observed against untreated controls.

Summary of results

Results from the wound simulation model indicate a statistically significant increase in the expression of Caspase-14 and hyaluronic acid in the wounded tissues treated with 2% BFEs. By significantly increasing the expression of Caspase-14 and hyaluronic acid in the tissue models, the BFEs are able to strengthen the barrier functionally by influencing structural proteins and cellular moisturization.

Figure 1: Hyaluronic acid expression from the wound simulation model (Green – hyaluronic acid; Blue – cell nuclei). Tissues treated with 2% BFE show a statistically significant increase in the hyaluronic acid production vs. untreated tissues.

2% Bioactive Fermentation Extracts
(Green – Hyaluronic acid; Blue Cell nuclei)



Untreated
(Green – Hyaluronic acid; Blue -Cell nuclei)

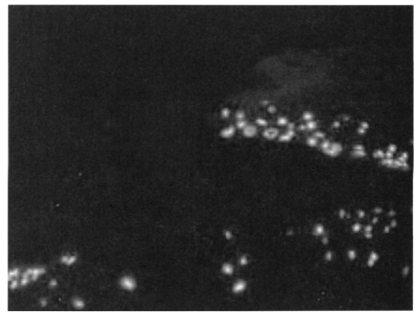
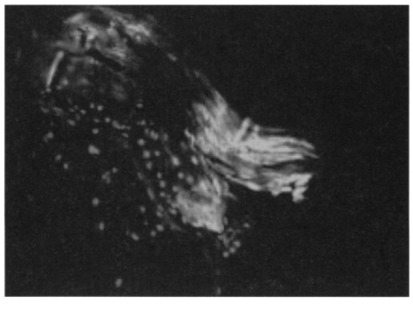
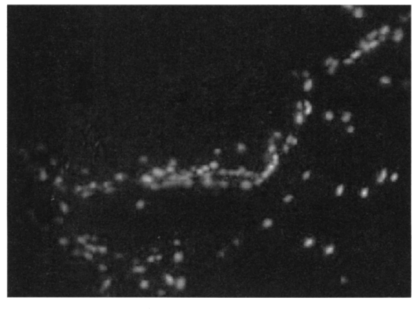


Figure 2: Caspase-14 expression from the wound simulation model (Red – Caspase-14; Blue – cell nuclei). Tissues treated with 2% BFE show a statistically significant increase in Caspase-14 production vs. untreated tissues.

2% Bioactive Fermentation Extracts
(Red – Caspase-14; Blue Cell nuclei)



Untreated
(Red – Caspase-14; Blue Cell nuclei)



Conclusion

BFEs provide a natural, safe, non-irritating and efficacious topical treatment for enhancing barrier formation at the cellular level. *In vitro* assay demonstrates that BFEs provide a way to accelerate barrier formation in compromised skin due to a physical insult, i.e., a wound. BFEs are unique new ingredients that can help improve the overall well-being of compromised skin. Our research proves that microorganisms with well-known properties can be grown in a competitive environment to generate either new activity, potentially new modes of action or new targets for topical treatments. We have demonstrated that targeted research can be predicted to yield desired skin care functions if the parameters for product development are well established.

MAPPING SDS PERMEATION AND INTERACTION WITH STRATUM CORNEUM LIPIDS BY VIBRATIONAL SPECTROCOPY

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[1]*Johnson & Johnson Consumer Companies, Inc., Skillman, NJ*
[2]*Department of Chemistry, Rutgers University, Newark, NJ*

Introduction:
In formulating personal care cleansers it is important to understand how surfactant interacts with the skin. The skin barrier is primarily provided by the lamellar lipid matrix of the SC, which consists of ordered lipids packed in orthorhombic and hexagonal domains. Surfactants are found to permeate into skin and are suggested to diminish the barrier properties of the SC by disrupting the lipid structural organization. The effect of surfactants on skin properties is well studied, properties such as skin barrier, transepidermal water loss, conductance, mechanical properties, irritation, and structural features by various imaging techniques[1,2,3,4]. These changes in skin properties are largely due to molecular interactions of surfactants with skin compounds; these molecular interactions are more difficult to study. Vibrational spectroscopy can be used to gain molecular information of skin components[5,6].

In the current work we map the permeation of the anionic surfactant sodium dodecyl sulfate (SDS) through skin with vibrational spectroscopy. We investigate the effects of SDS on SC lipid packing, phase behaviour, and chain conformational order using FT-IR spectroscopy. The use of acyl chain perdeuterated SDS allows unequivocal spectroscopic detection of both endogenous lipid and exogenous material in intact tissue. Additionally, we also evaluate how the non-ionic surfactant PEG-80 sorbitan laurate, long used to create more tissue compatible cleansers[7], reduces SDS interaction with the skin.

Experimental
Excised full thickness human skin was topically treated with 120 μL 12.5 mg/mL SDS-d25 (5xCMC) for 40 hours at 34°C. The thickness of skin samples were ~2-3mm and the diameter of the area exposed to SDS-d25 treatment is 8 mm. After treatment, skin samples were microtomed to 7 μm thin slices perpendicular to skin surface and evaluated by FT-IR imaging in transmission mode with a pixel size of 6.25 x 6.25 μm^2. Additionally, transmission IR spectra were taken from isolated SC from 6°C to 90°C using approximately 3°C increments. Solution properties are also studied; surfactant dynamics are determined by pendant drop Tensiometer, and surfactant micelle sizes are determined by light scattering.

Results & Discussion:
IR spectra were acquired as a function of temperature from isolated human SC exposed to SDS for various incubation periods at 34°C. SDS reduces the amount of orthorhombic phase in the SC and increases the amount of disordered lipid, which is consistent with the observed decrease in the lipid Tm (acyl chain melting temperature).

The spatial distribution of SDS concentration was obtained by infrared spectroscopic imaging from full thickness human skin. In Figure 1a, a light microscope image of the skin slice is shown with the skin surface, SC, positioned to the left followed by epidermis and dermis to the right. Different skin sections (i.e. stratum corneum, viable epidermis and dermis) can be approximately differentiated in the visible image. Figure 1b shows the SDS concentration distribution in the skin, as determined from the peak intensity of the CD2 stretching bands between 2050-2250 cm-1. With extinction coefficient determined for the CD2 stretching bands from aqueous and alcoholic SDS-d25 solutions, SDS-d25 concentration in skin was determined and is shown in logarithm scale in the lower left figure. Highest SDS-d25 concentration was observed in stratum corneum region and decrease sharply going into the viable epidermis. SDS-d25 was able to diffuse into dermis and a significant concentration drop was also observed at the dermis/epidermis boundary. Also there is significant lateral concentration differences, these SDS concentration differences map to the visual image and corresooind to latreal differences in the thickness of the SC. Figure 1c shows the SDS-d25 distribution in skin is presented in 3D space with logarithm of SDS-d25 concentration as the vertical axis.

We also explored how the non-ionic surfactant PEG-80 Sorbitan Laurate alters the solution properties of SDS. Dynamic light scattering is used to quantify the micelle size of different surfactant systems. The rate of surfactant arriving at the air/water interface is studied with dynamic surface tensiometry. We observe that these mixed micelles, including ethoxylated non-ionic surfactant, have slower surfactant dynamics. In two in vitro measures, the addition of non-ionic surfactant is shown to improve the tissue compatibly of the surfactant system. Dye leakage across a monolayer of epithelial cells shows less disruption of the tight junctions between cells with the addition of the non-ionic surfactant. We also observed a significant reduction in inflammatory cytokine released by a tissue culture model with the addition of the non-ionic surfactant. Additionally, we observe the corresponding improved tissue compatibility in an in vivo model.

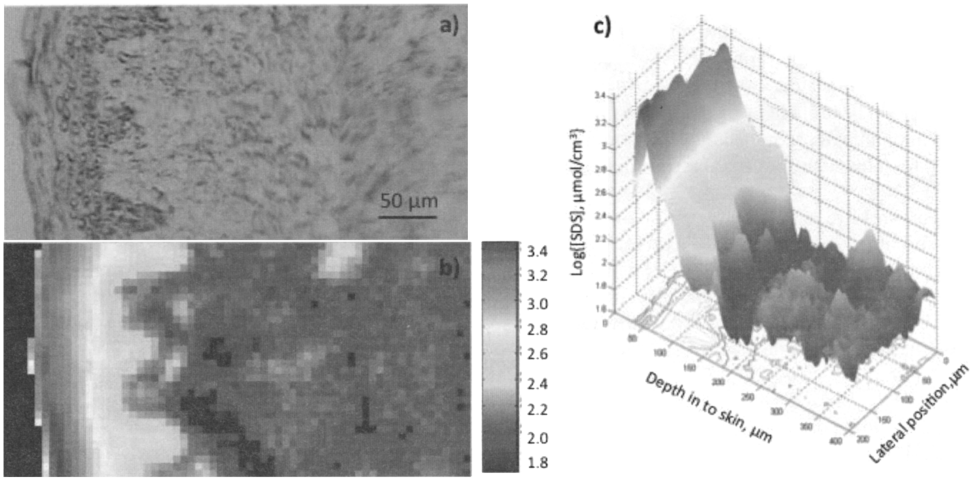

Figure 1. a) light microscopy of full thickness skin, with SC at left, b) concentration map of SDS in skin from IR, c) SDS concentration depth profiles in the skin at each lateral position.

Conclusions:

In this study we elicit some of the molecular details of how this surfactant interact with SC lipids. Raman spectroscopy and IR imaging are able to track SDS permeation across different regions of skin. Exposure of SC to SDS results in less ordered SC lipids. SDS penetrates into the SC and participates in the molecular ordering of SC lipids, suggesting that SDS is not in a micellar state in the skin. PEG-80 sorbitan laurate co-micellizes with SDS, changing the micelle dynamics and reducing the SDS penetration into skin.

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THE SIGNIFICANCE OF GENE EXPRESSION FOR THE FORMULATING COSMETIC CHEMIST: PRACTICAL APPLICATIONS TODAY AND ADVANCES IN THE FUTURE

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Are genetically designed skincare products the ultimate approach to creating the most effective skin care treatments? Clinical evidence indicates that polyphenols have health benefits, but perhaps not as antioxidants. The answers to these questions are likely to be found in the study of gene expression, more specifically in the study of epigenetics. Genomics is defined as the study of all the genes of a cell, or tissue, at the DNA, mRNA or protein level. Epigenetics is the study of heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence. The implication of recent epigenic observations is that we have the capability to improve the appearance of aging skin through epigenic therapy, by altering the gene expressed instructions to targeted skin cells. During this presentation gene expression data for three skin care products will be reviewed with a "look to the future" of potential new skin care product development.

APPLICATION OF RICE MERISTEM CULTURES TO HUMAN SKIN PROMOTES REJUVENATION AT THE EPIGENETIC, PROTEIN AND MACRO LEVEL

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Abstract

A red rice meristem culture was found to deliver benefits at the epigenetic, protein and macro *in vivo* level. We culture meristem cells of *Himalayan Red Rice*. These cells are grown in a bioreactor and subjected to an ozone stress which causes them to produce secondary metabolites. Through an *in vitro* assay we show rejuvenation at the epigenetic level of treated cells and an upregulation of the key protein collagen. An *in vivo* assay found macro level benefits to the skin.

DNA Methylation as a Type of Epigenetic Modification

Epigenetics is the study of heritable changes in gene expression that is caused by a mechanism other than changes to the DNA sequence. Epigenetic factors and changes have been shown to play an important part in cellular differentiation, development, aging and disease. DNA methylation in regards to epigenetic changes occurs at the cytosine-5 in **CpG** dinucleotides. The **p** represents the phosphate that links the **C**ytosine and **G**uanine together. This helps differentiate CpG from a CG base pair. Methylation of the CpG-rich promoter areas of genes has been identified as an essential mechanism in the regulation of gene transcription[1, 2]. For humans, promoters tend to gain methylation with age. This trend can be seen when viewing the variation in methylation in regards to aging and environmental exposure to toxins such as exposure to smoking, arsenic or asbestos.

Epigenetics can be thought of as a "youth switch". The switch is turned on in young cells and turned off as cells age. DNA methylation could also be considered as a stop sign in gene promoters (Fig. 1). Young healthy cells are thought to maintain their CpG sites in a low methylated state to allow transcription to occur. Less methylation is fewer stop signs to prevent the gene from being expressed. As aging occurs and DNA methylation drift increases the amount of DNA promoter methylation, the gene is expressed less causing lower protein production. Undifferentiated plant cells may help reverse promoter methylation. There may be various plant transcription factors, cellular components and homologous proteins and peptides that may be able to be applied to aged skin and help the skin cells to rejuvenate by decreasing promoter DNA methylation. By profiling the CpG methylation differences in young and aged skin tissues, characterization of the role of aging related to methylation variation can be observed.

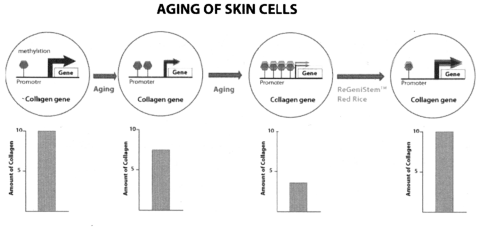


Figure 1. As cells age, there is an increase in promoter methylation, leading to a decrease in gene transcription and protein expression. The application of elicited undifferentiated red rice meristem cultures may contain various cellular factors that can help modulate promoter DNA methylation and rejuvenate the cells

In Vitro Efficacy Studies

Global Decrease of CpG Methylation in Gene Promoter Regions

Red rice meristem culture was evaluated using the **D**NA **C**pG methylation chip (Agilent Technologies). The evaluation of global CpG methylated sites involved a two-step process wherein three sets of human dermal fibroblast cultures were intrinsically (cell culture passage) and extrinsically (UVB light exposure) aged. These cells were divided into three groups and then treated as follows:

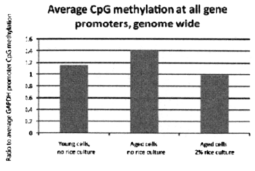
A. Control (Young Cells): untreated cells, no UVB
B. Control (Old Cells): untreated cells, extrinsically and intrinsically aged
C. Treated Cells: treated with 2% red rice meristem culture, extrinsically and intrinsically aged

The resulting data consisted of three sets of approximately 250,000 CpG sites each. Since we were interested in the change of methylation at the promoter level, all CpG sites within all promoters were analyzed (Graph 1). As expected, there is an increase of global methylation at the promoter level when comparing young cells to old cells, both without red rice meristem culture. The application of 2% red rice meristem culture to old cells had the ability to modulate the epigenetic environment and decrease the global level of promoter methylation, perhaps helping the old cells to function and perform as a younger, healthier cell.

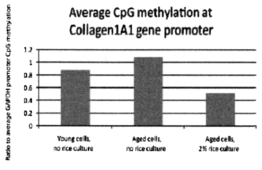
CpG Methylation at Collagen 1A1 (COL1A1) Gene and Pro-collagen 1 Protein Synthesis

When a specific gene, COL1A1, was examined for its promoter methylation levels, the resulting data showed that treatment with 2% red rice meristem culture also reduces CpG methylation at the COL1A1 promoter region on treated aged cells (Graph 2).

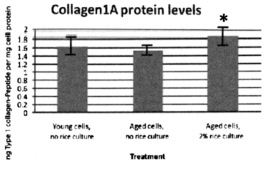
To correlate DNA demethylation to Collagen 1A synthesis, a protein assay was conducted on human fibroblasts treated with and without 2% red rice meristem culture. The results shown in Graph 3 confirm that treatment with 2% red rice meristem culture increases collagen synthesis in cells when compared to old untreated cells.



Graph 1. Average Global CpG methylation at the promoter region with red rice meristem culture treatment



Graph 2. Average CpG methylation at the COL1A1 gene promoter



Graph 3. Protein assay showing the effect of red rice meristem culture on collagen synthesis

***In Vivo* Efficacy Studies**

The efficacy of the red rice meristem culture was evaluated via a 60 day *in vivo* study which examined the ingredient's effects on skin hydration, skin barrier function and pore size reduction. Placebo, 2% and 4% red rice meristem culture were tested on a total of 60 panelists with application of the product twice per day. The results demonstrate a dose dependent increase for all three parameters at the end of the 60 day study. In addition to instrumental analysis and professional clinical evaluation of the subjects' skin, clinical photographs demonstrate improvements in skin tone, wrinkle reduction and fine line reduction (Figure 2).

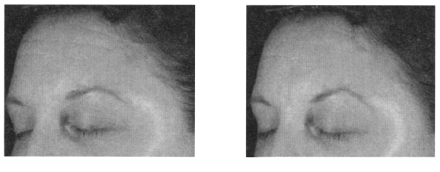


Figure 2. The photographs show a panelist who used the product containing 2% red rice culture. There is a noticeable reduction in the depth of the wrinkles on her forehead from day 0 (left photo) and day 60 (right photo).

Conclusion

Growing a red rice meristem culture in bioreactors is a sustainable process that reduces residual biomass waste, but the benefits of this technology are not limited to environmental impact. Through elicitation of our red rice meristematic cell cultures, we were able to produce appreciable quantities of secondary metabolites in the culture. When the culture was tested on fibroblasts *in vitro,* we showed a reverse in the age related increase of methylation in the promoter region of genes. This rejuvenation at the epigenetic level may have multiple benefits to the cells and to the skin. Some benefits include anti-aging, the renewal of gene and protein expression levels to that found in younger cells, such as was shown for collagen and the reversal of age-related decreases in protein production. In essence, the skin, through an application of red rice meristem cultures, is pushed to a younger, healthier state. The red rice meristem culture was also able to improve pore appearance and improve the skin barrier function; two macro effects that were seen from the *in vivo* study.

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EFFECT OF A ACTIVE INGREDIENT ON THE EXPRESSION OF BARRIER FUNCTION-RELATED GENES AND MOISTURISATION-RELATED PROTEINS

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OBJECTIVE

Proper skin barrier function and moisturisation result from appropriate histological structuring and cell-cell communications at both the epidermal and the dermal levels and between these two tissues. More precisely, the establishment and the maintain of the different inter-keratinocyte junctions as well as that of the cornified layer are of a great importance for the skin to ensure its protection function. Among the different kinds of junctions, the presence of aquaporins, and particularly that of one of their main components, aquaporins-3 (AQP-3), has been recently reported as essential.

Xylityl glucoside (XG) is a moisurizing active ingredient, which efficacy had been clinically proven. Its mode of action had been partially elucidated, our previous results having shown that it was able to increase both the production of glycosaminoglycanes (which are considered as "water reservoirs" in the dermis) in normal human fibroblasts and that of ceramides in the epidermis.

The aim of this study was thus to further investigate its regulation properties, within the epidermis or their main cell components, *i.e.* keratinocytes, on the genes or proteins involved in skin barrier and moisturisation functions.

METHODOLOGY

A blind transcriptomic screening was performed on reconstructed epidermis (REp) which were composed of normal human keratinocytes (NHK) and which were topically treated with a XG containing-formulation (3%) (*vs.* placebo-treated REp). This study was carried out by quantitative (q) reverse transcription (RT)-polymerase chain reaction (PCR) analysis of the effects on epidermal differentiation and barrier function related genes using low density TaqMan (Straticell, including housekeeping genes). Pathway analysis of XG-induced gene regulations was performed with the GeneSpring® software (Agilent).

In parallel, a protein screening was performed on NHK by both ELISA experiments against hyaluronic acid (supernatents) and western-blot against AQP-3 (cell lysates). Analysis was performed respectively 24h and 48h after the treatment with XG (0.01%; *vs.* untreated cells) or with the reference molecule retinoic acid (RA, 0.2 μM). Normalization was performed, respectively, on protein contents (BCA method) and on β−actin expression.

RESULTS

First, as expected, the qRT-PCR screening confirmed that XG, when topically applied on REp, was able to induce an increase in the level of expression of epidermal differentiation and barrier-function related genes in comparison with placebo formulation-treated REp. Fold-change and pathway analyses showed that XG targets included genes encoding proteins involved in keratinocyte-keratinocyte junctions (claudins, corneodesmosin, AQP-3…), enzymes and structural components of the cornified layer (transglutaminases; loricrin…), as well as epidermal differentiation (involucrin, kallikreins…). Unsurprisingly, most of these genes belonged to the famous epidermal differentiation complex 1q21, including less known genes, such as repetin (Figure 1).

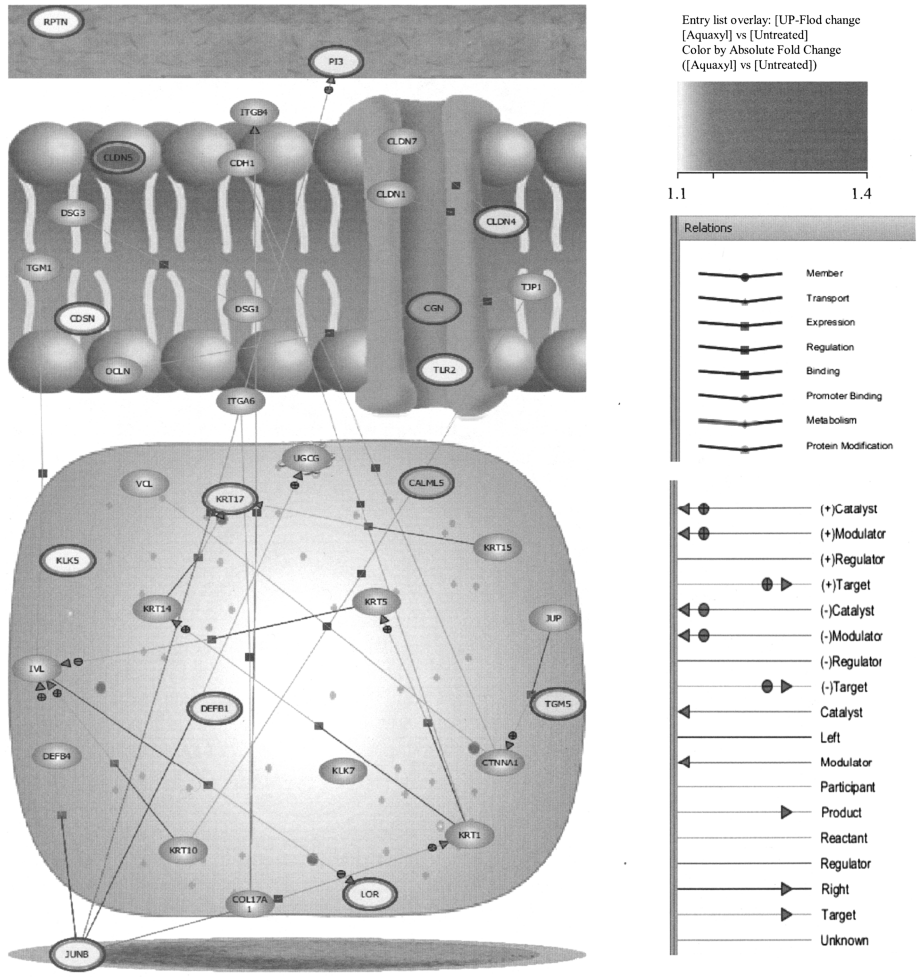

Figure1: Pathway analysis obtained with the GeneSpring® software (Agilent)

Western-blot analysis showed that XG was able to induce a moderate increase (9%) in the expression of AQP-3 when added to the culture medium of NHK, while RA showed an effect of 49%. Moreover, XG induced an increase of 31% of hyaluronic acid production, while RA showed an effect of 132%.

CONCLUSION

In conclusion, taken together, these results show that XG is able to regulate, at both gene and protein levels, the structural components and the metabolic regulators involved in epidermal differentiation. They also enlighten XG ability to regulate the main skin barrier function-related networks. Indeed, despite the differences between the two models (respectively 3D and 2D ones) and between their respective treatment (topical application *vs*. culture medium), XG was able to regulate, at least partially, the same skin barrier and moisturizing function-related targets. In the future, further investigations on protein expression will be required to confirm its action on all these newly identified targets.

GLYCATION AND GLYCOTOXINS IN SKIN: INHIBITION AND REVERSAL?

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INTRODUCTION:

Today's "fast foods" provide so-called fast sugars (glucose, fructose) which enable the mitochondria to produce the additional energy the body needs. Unfortunately, some sugars can bind directly to cell or tissue proteins and/or become oxidized glycotoxins, highly reactive free radical metabolites. Glycation is an unspecific non-enzymatic reaction of sugars and their radical derivatives with proteins, leading to reticulation, AGEs (Advanced Glycation Endproducts) and other glycotoxins. Glycation of mitochondrial proteins reduces their ability to produce energy, and long-lasting structural proteins such as collagen and elastin lose their ability to respond to mechanical stresses.

OBJECTIVE:

The objective of our work was to study and understand further details of the glycation process in the skin as well as the mechanisms of action in inhibiting or even reversing the reactions.

METHODS: culture of human fibroblasts; use of 3D skin explants; ELISA and immunefluorescence tagging, Western Blots, measurements of melatonin in presence and absence of methylgyoxal, AGEs *in vitro* and *in vivo*, enzyme kinetics (glyoxalase, proteasome); clinical tests include AGE-Reader™ measurements, analysis of DSquame-strippings and measurement of skin structure with Reviscometer™ (acoustical wave propagation).

RESULTS:

It is important to understand that in all following cellular and skin model experiments, a pre-incubation period of 5 days of the cells and/or the skin with methylglyoxal preceded the contact with a control medium or the *Albizia* containing medium. Thus, glycation and the catalytic production of AGEs had already been set in motion before any preventive and/or curative action of the plant extract was initiated. This is a more challenging and more reality-relevant protocol than simultaneous incubation of cells or skin with MGO (the "aggressor") and the active ingredient (the "protector").

***In vitro* data:**

A plant extract of *Albizia julibrissin* (called the "night sleeper" in Persia) was prepared by lixiviation from the leaves of this tree. In screening assays it turned out that this particular extract inhibited the glycation of BSA (bovine serum albumin) with glucose by close to 50% and that it maintained fibroblast proteasome activity at base level compared to BSA-AGE affected cells (-16%).

From this initial observation, further studies were undertaken to investigate the mechanisms and efficacy of anti-glycation activity. Levels of melatonin, the sleep-regulating hormone and, in particular, cellular energy preserver in the skin [1] are maintained in human skin fibroblasts. Carboxymethyl-lysine (CML) and Carboxyethyl-lysine (CEL) are typical AGE products of collagen ageing and glycation *via* a reaction with methylgyoxal (a first oxidized derivative of sugar). Treating fibroblasts in culture with MGO leads to a strong reduction in cell viability (-44%) and a huge increase in CML (+124%, $p<0.01$). When the cells are then in contact with the *Albizia* extract for five days and CML levels are again checked in comparison to untreated cells, they come down by about 43% in the *Albizia* contacted cells.

***Ex vivo* data on explants**

Human abdominal skin explants from a 34-year-old woman were placed in contact with MGO dissolved in the survival medium. After the glycation phase the skin (n=5/case) received an application of a cream containing 4% of the *Albizia* extract preparation or the placebo cream (both of which were used in the following clinical tests). Topical applications were administered once per day for 4.5 days. The skin sections were cut with a microtome before being labeled with a fluorescent anti-AGE antibody. Ten photos/case were taken and analyzed on image analysis software. The results show that AGE labeling was

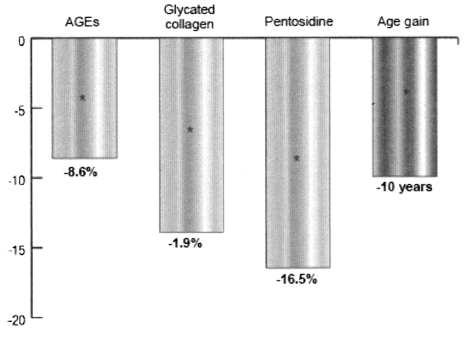
	Concentration	AGEs (AFU*)	% change; significance
Explants ; n=5	MGO then Placebo cream	9.56 ± 1.30	Reference
	MGO then 4% cream	7.33 ± 1.40	-23.4%; *p<0.01*

** AFU = Arbitrary fluorescence unit*

located particularly in the dermis, an area rich in stable matrix proteins. Quantifications were therefore carried out in this area.

This experiment allows us to conclude that the *Albizia* extract reduces MGO catalyzed AGE formation *via* upstream blockage of glycotoxin formation. This is confirmed by the quantification of glyoxalase-1 synthesis in these MGO treated fibroblasts. A Western blot analysis shows a 56% drop in detoxifying glyoxalase-1, whereas the *Albizia* treated cells maintain their initial glyoxalase-1 level.
Just like the proteins with a long half-life such as collagen, proteins with a much shorter half-life can be quickly altered by glycotoxins. Vimentin plays a vital role in the organization of contraction and cohesion of the dermis. This protein is also known to be sensitive, particularly to MGO, which forms CML [2]). It is therefore important to protect it. The same explants as for the previous experiment were used. Vimentin labeling was performed using a fluorescent anti-vimentin antibody and the same methods were used for quantification. MGO incubated and then vehicle treated skin samples showed a drastic reduction in Vimentin, whereas *Albizia* extract prevented this effect and maintained Vimentin staining 75% higher ($p<0.01$).
The detrimental effects of glycation are numerous and affect not only the structure of cells and their components, but also their function. An important property of fibroblasts is to be able to latch on to collagen fibers *via* adhesion sites. In a classical collagen gel contraction experiment, the MGO induced loss of contractile force of 78% is, once more, prevented by the incubation (post-MGO-treatment) of the cells. Further experiments demonstrated the "anti-fatigue" activity of the *Albizia* preparation along the lines of Remor et al. [3] protocols, such as fatiguing the cells by MGO treatment or by inducing ATP deficiency and then monitoring contractile potency with a CCD camera.

***In vivo* studies**: 44 panelists with stressed skin (as determined by AGEs present in adhesive strippings) were enrolled in randomized vehicle controlled clinical trials on three subgroups. Endpoints measured were auto-fluorescence (AGE-Reader™), AGE content in tape strippings of the forearm, skin fatigue analysis using the Reviscosimeter™ method and a daily and weekly self-assessment of facial fatigue every morning. Creams with either 2% (day cream) or 4% (night cream) of the *Albizia* preparation were tested against the identical vehicle without the plant extract. Application sites were the two forearms, frequency of application was twice a day over a two month period.
The following graph indicates the difference in AGE-Reader™ Autofluorescence (AF) values between the *Albizia* treated skins and the vehicle treated ones, after 2 months. AF values are converted into AGEs, glycated collagen or Pentosidine according to these authors [4-7] and can further be interpreted in age differences based on a large data file linking AF values to skin age.



Similar significant results were obtained with the Reviscosimeter method. Self-assessment (scoring of various facial features such as baggy eyes, dark circles, drawn faces, every morning at wakening, in front of a mirror), established the visible benefits of a novel cosmetic ingredient to combat aging and skin fatigue both *via* preventive and curative treatment of glycation, and confirmed that *Albizia* is not without reason called "night sleeper", as skin treatment with this extract appears to give similar results as a good "beauty sleep".

CONCLUSION

The examples presented show, on the basis of the extensive *in vitro, ex vivo* and clinical data, that this approach to simultaneous immediate skin beautifying and long-term physiological skin care is possible and justifiable. The measurable results on the three panels of volunteers are in good correlation with the proposed *in vitro* mechanisms of activity of the ingredients formulated in these make-up preparations.

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INNOVATIVE FORMULATION APPROACHES TO DELIVER HAND SANITIZERS WITH DESIRABLE AESTHETICS AND BENEFITS

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Studies have shown that the use of alcohol hand sanitizers can significantly reduce the transmission of infectious diseases (1-3). These products will become increasingly valuable in the coming years as the threat of infectious disease continues to grow, due to the changing nature of pathogens and our own resistance to infection (3). Consumers have numerous choices between existing products on the market, though few of these are innovative. As hand sanitizers become an essential part of hygiene maintenance, our industry is looking for the next generation in hand sanitizer formulations.

Using innovative approaches, we have identified new formulation methods to create hand sanitizers with unique aesthetics, as well as interesting product forms compatible with benzalkonium chloride. Whereas alcohol-based hand sanitizers are highly effective, developing alcohol-free formulations is also beneficial since they are non-flammable, and alleviate concerns of skin drying (4). Our optimized formulations include pearlescent, lotion, and foaming types of systems.

Pearlescent Hand Sanitizers:

Stearoxytrimethylsilane readily undergoes hydrolysis at pH below 6 to form stearyl alcohol, which achieves a pearlescent effect in hydroalcoholic systems (5). The pearlescent appearance can be controlled to provide varying degrees of opacity from a metallic pearlescence to opaque. Design of experiments was used to investigate the effects of stearoxytrimethylsilane concentration, thickening polymer type and concentration, and formulation processing parameters on the properties of pearlescent hand sanitizers.

In order to evaluate the effect of stearoxytrimethylsilane concentration, and thus stearyl alcohol concentration, in hand sanitizer formulations, a standardized hand sanitizer was formulated at three different stearoxytrimethylsilane concentrations: 1.5 wt %, 2.0 wt %, and 3.0 wt %. The size and quantity of stearyl alcohol particles produced was visually evaluated in the standardized hand sanitizer formulations. Smaller particles in a high quantity were observed in formulations with 1.5 wt % and 2.0 wt % stearoxytrimethylsilane, resulting in a pearlized and shimmery appearance. The formulation with 3.0 wt % stearoxytrimethylsilane contained fewer, large particles and had an appearance of heavy shimmer.

The effect of AMPS-based rheology was also evaluated in a standardized hand sanitizer formulation. The polymer chemistry and concentration were varied. The concentration of stearoxytrimethylsilane was kept constant at 1.0 wt %. Polymers evaluated include: Ammonium Acryloyl Dimethyltaurate/VP Copolymer (AMP-S-VP), Ammonium Acryloyl Dimethyltaurate/ Carboxyethyl Acrylate Copolymer (AMP-S-CEAC), and Ammonium Acryloyldimethyltaurate/ Beheneth-25 Methacrylate Crosspolymer (AMP-S-Beheneth-25). It was found that the degree of pearlescence varied greatly depending upon the chemistry of the polymer used and the concentration of polymer. Pearlescence of final formulations varied from a metallic luster to an opaque cream. Hand sanitizer formulations with a traditional AMPS-based rheology modifier, AMP-S-VP, were formulated at concentrations of 0.9 wt %, 1.0 wt %, 1.5 wt % and 2.0 wt % polymer. Pearlesence of the formulations ranged from a pearlized shimmery appearance at lower polymer concentrations, to high shimmer at 2.0 wt % AMP-S-VP. AMP-S-CEAC, an acrylates-modified AMPS polymer, at 2.0 wt % provided similar appearance to AMP-S-VP at 1.0 wt %. The viscosity of the AMP-S-CEAC formulation was significantly less, 10,000 cP vs. 65,400 cP, respectively. AMP-S-Beheneth-25, a hydrophobically-modified AMPS polymer, at 1.0 wt % provided a pearlized opaque appearance with high viscosity of 70,700 cP.

Benzalkonium Chloride Lotion Hand Sanitizers

Typically, lotion formulations contain anionic emulsifiers as a primary component. In considering hand sanitizer formulations where benzalkonium chloride is the desired active ingredient, anionic emulsifiers were not available due to electrostatic interactions between the cationic active and anionic emulsifier, which would result in instability. In our alternative approach, benzalkonium chloride hand sanitizing lotion was formulated using amodimethicone glycerocarbamate as a water-in-silicone emulsifier. The lipophilic and hydrophilic functionalities of amodimethicone glycerocarbamate stabilized the emulsion and made it possible to formulate a lotion with benzalkonium chloride. As discussed, benzalkonium chloride is not compatible with the anionic emulsifiers typically used in lotions due to its charge, however we found that it can readily be incorporated into a silicone-based formulation. Incorporation of benzalkonium chloride in the water phase was critical for hand sanitizer lotion stability. The lotion has desirable aesthetics for skin feel and appearance due to additional silicones used as emollients in the formulation.

Benzalkonium Chloride Foaming Hand Sanitizers

A surfactant-free benzalkonium chloride foaming hand sanitizer was developed based on polyethylene glycol (PEG). The PEG stabilized the air-water interface and provided a dense and stable foam when dispensed from a mesh-pump nozzle. Design of experiments was used to evaluate multiple polymers with different degrees of ethoxylation, charge character, and concentration. Polymers evaluated included: PEG-6, PEG-8, PEG-32, PEG-75, and Polyquaternium-43. Polymer solutions in water were evaluated based on clarity, foam stability, and skin feel. Visual observations of clarity and foam stability were made to determine the ideal polymer and concentration for a clear, foaming formulation. Generally, it was determined that polymer chemistry and concentration were important in order to deliver a clear formulation with foam comparable to formulations based on cationic and amphoteric surfactants. PEG-6, at 5.0 wt %, delivered a clear system which produced stable voluminous foam. The order of addition of cationic ingredients was critical for formulation stability. The size and density of the foam bubbles from the PEG-based hand sanitizer formulation was compared to that of market standards, and it was observed that more uniform foam was generated in the PEG-based hand sanitizer. The mildness of benzalkonium chloride and polyethylene glycol allows this formulation to be a gentle hand sanitizer compared to current technology of cationic or amphoteric surfactants.

Efficacy Data

Efficacy of the Pearlescent Hand Sanitizer, Benzalkonium Chloride Hand Sanitizing Lotion, and Benzalkonium Chloride Foaming Hand Sanitizer was tested using the Time Kill Test based on ASTM E2315 "Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure." Pearlescent Hand Sanitizer met the acceptance criteria for Disinfectant/Antiseptic at the 30 second time limit. Benzalkonium Chloride Hand Sanitizer Lotion and Benzalkonium Chloride Foaming Hand Sanitizer met the acceptance criteria for Disinfectants/Antiseptics at the 1 minute time limit. All results were comparable to marketed products.

	Organisms	Inoculum Recovery	Log Reductions		
			30 seconds	1 minute	3 minutes
Pearlescent Hand Sanitizer	*Pseudomonas aeruginosa* ATCC # 15442	3.3×10^6 $\log_{10} = 6.52$	≥6.52	≥6.52	≥6.52
	Staphylococcus aureus ATCC # 6538	1.1×10^6 $\log_{10} = 6.04$	≥6.04	≥6.04	≥6.04
Benzalkonium Chloride Lotion Hand Sanitizer	*Pseudomonas aeruginosa* ATCC # 15442	3.3×10^6 $\log_{10} = 6.52$	≥6.52	≥6.52	≥6.52
	Staphylococcus aureus ATCC # 6538	1.1×10^6 $\log_{10} = 6.04$	4.57	≥6.04	≥6.04
Benzalkonium Chloride Foaming Hand Sanitizer	*Pseudomonas aeruginosa* ATCC # 15442	3.3×10^6 $\log_{10} = 6.52$	3.86	≥6.52	≥6.52
	Staphylococcus aureus ATCC # 6538	1.1×10^6 $\log_{10} = 6.04$	≥6.04	≥6.04	≥6.04

Table 1. Efficacy of hand sanitizers as tested using Time Kill Test based on ASTM E2315

Conclusion

Hand sanitizing products have become ubiquitous in our society. The product forms are efficacious, but lack innovation and excitement for the consumers. Hand sanitizing products are ready to move to the next generation, maintaining efficacy while delivering superior aesthetics and benefits to consumers. Using new ingredients or existing ingredients in unique ways provides increased formulation options for non-traditional product forms.

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UNDERSTANDING THE INFLUENCE OF EMULSIFIERS, EMOLLIENTS AND ADDITIVES ON LAMELLAR PHASES IN COSMETIC EMULSIONS

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Lamellar phases, formed by combinations of emulsifiers and consistency modifiers such as Stearyl Alcohol or Glyceryl Stearate, are classical tools for stabilizing cosmetic O/W emulsions. Emulsion properties like appearance, texture, sensory aspects, water resistancy, high temperature stability and overall robustness against critical additives depend very much on the nature of these liquid crystalline structures.
The formation of liquid crystalline networks in emulsions has systematically been studied for a new PEG-free O/W emulsifier (Polyglyceryl-3 Dicitrate/Stearate) and a classical ethoxylated O/W emulsifier (Ceteareth-25) Moreover, the influence of different co-emulsifiers and various types of oils has been examined.

Oscillatory rheological measurements, Differential Scanning Calorimetry (DSC), Small Angle Neutron Scattering (SANS), PFG-NMR measurements and Transmission Electron Microscopy (TEM) have been used to investigate systematically the influence of emulsifiers, emollients and additives on the formation of lamellar networks in emulsions. Especially the use of neutron scattering is a powerful tool for the examination of bilayer structures in cosmetic emulsions, as methods like light scattering or light microscopy are not suitable for the detection of liquid crystalline structures in emulsions that have a domain size that is smaller than 500 nm (as is the case in most cosmetic emulsions).

It can be shown that Polyglyceryl-3 Dicitrate/Stearate forms liquid crystalline gel structures (bilayers) in water even if no additional consistency enhancers (e.g. Stearyl Alcohol and Glyceryl Stearate) are added. This finding explains why this emulsifier can be used without additional consistency enhancers for manufacturing of O/W lotion systems. Upon addition of consistency enhancers, the bilayer structure remains intact but the overall rigidity of the bilayer is increasing. Moreover, the studies revealed that the presence of oil droplets is an essential requirement to obtain a significant viscosity build-up in emulsions. Ceteareth-25 forms basically similar bilayer structures when combined with fatty alcohols. The formation process of the bilayers and the structures in the final emulsions are comparable to the Polyglyceryl-3 Dicitrate/Stearate system. However, it can be demonstrated that the PEG-free emulsifier system is far more robust and versatile when oil phases of different polarities are used.

O/W creams stabilized by combinations of emulsifiers and consistency enhancers consist of oil droplets and liquid crystalline bilayer structures in the aqueous phase (e.g. vesicles). O/W creams are not thickened by the pure presence of liquid crystalline bilayer structures in the emulsion. A significant amount of additional oil is needed in order to obtain a cream-like consistency. As a direct consequence of these studies, a more realistic model can be proposed for the actual structure of liquid crystalline phases in cosmetic O/W emulsions and more efficient predictions can be made about the influence of various ingredients on emulsion properties.

DEVELOPMENT OF A NOVEL, SOOTHING TISSUE INCORPORATING PHASE CHANGE MATERIALS

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Background

During normal, infrequent, everyday usage, tissue products are inherently nonirritating. However, during allergies, a cold, or a flu event, the combination of frequent and repetitive usage of tissue products can lead to inflammation of the nasal area (1). Several products are available on the market to alleviate wiping-induced irritation by leveraging softer tissue, a lotion coated tissue, or aromatherapy in the form of a mentholated tissue. The technologies employed are effective at minimizing and repairing barrier damage to the nose, or making the consumer feel better emotionally, but none address soothing an already red and irritated nose. One approach would be to deliver a cooling sensation to the warm and irritated nose. The limitation to most conventional cooling products is that they tend to employ volatile solvents, such as ethanol, or neurosensory agents. Neither approach is particularly applicable to dry facial tissue products. A phase change material (PCM) offers a more attractive approach as it is a solid at room temperature and provides a cooling sensation by taking advantage of a high latent heat of fusion as it melts (2).

Success Criteria

The design of this formulation centered around four technical parameters; 1) provide a noticeable cooling sensation upon use, 2) provide an improved soothing benefit compared to a market standard (3), 3) have an equivalent safety profile to a market standard, and 4) be utilized on current production assets. While delivering against these objectives, the new formulation must meet and maintain performance characteristics of current lotion tissue such as strength, absorbency, consumer acceptance (hand-feel, residue left on skin), packaging compatibility, and of course, cost. The later criteria will not be discussed here.

Formulation Development

Initial selection of PCMs centered on INCI registered ingredients that are solids at room temperature, have a melting point at or near body temperature, a large heat of fusion, and availability. With these criteria in mind, the list of candidates quickly narrowed to Octadecane and Stearyl Heptanoate. Differential Scanning Calorimetry (DSC) analysis of these ingredients gave melting points of 29.1^{o}C and 28.22^{o}C, respectively. The measured enthalpy of fusion for Octadecane was found to be 214 J/g while Stearyl Heptanoate was slightly lower at 177 J/g. These values compare well with reported data (4, 5). While meeting the thermodynamic criteria of the project, these ingredients used on their own did not provide the appropriate soothing benefit.

Diluting these pure PCMs with various fatty alcohols and fatty acids provided formulations with excellent thermodynamic performance. In addition, these diluted formulations showed reduced crystallite formation when coated on tissue. Evaluation of coated tissues by trained panelists confirmed that the perceived cooling sensation remained intact and the overall aesthetics were acceptable. Clinical evaluation of soothing properties was not as conclusive. However, it seemed clear that the crystallite size was a key factor in soothing performance as these iterations were better than the pure PCMs.

The final round of formulation modification focused on finding a second additive that could further reduce the size of the crystallites with minimal impact on the enthalpy of fusion. Of the second diluents tested, Polyethylene had the greatest impact on crystallite size while still delivering a melting point of 30.1^{o}C with an enthalpy of 168.6 J/g. Prototype tissues employing formulations utilizing Polyethylene and various fatty alcohols were then assessed against the remaining performance criteria. Compositions based on Polyethylene with either Stearyl Alcohol or Cetyl Alcohol were found by trained panelists to deliver the perceivable cooling sensation along with positive aesthetics. Evaluation of the impact on skin barrier properties of tissues with these cooling formulations showed equivalence when compared to marketed lotion tissue products. Based on all the evaluations, the combination of Polyethylene and Stearyl Alcohol was found to be the best performing code.

Finally, a full soothing panel (3) was placed comparing the prototype tissue against marketed lotion tissue controls. The prototype tissue was based on Stearyl Heptanoate as the cooling agent with Stearyl Alcohol and Polyethylene as diluents to provide a smooth coating of the phase changing composition on the tissue.

The PCM prototype tissue was found to be significantly more soothing by the panelists, with panelists displaying higher average soothing ratings 96% of the time versus the control lotion tissues.

Conclusion

By delivering a perceivable cooling sensation to the nose during use, the PCM prototype tissue was found to be significantly more soothing than marketed products. Completion of the remaining commercial development found this formulation to meet or beat all project success criteria and the product was successfully launched in the fall of 2011.

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A BREAKTHROUGH APPROACH TO LIP BALM SUNSCREEN FORMULATION: SAVOR THE FLAVOR

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Background & Objective

Human lips are particularly susceptible to damage from UVA and/or UVB radiation as they lack the severe burning of the lips which have little or no melanin. The longer UVA rays allow for relatively deeper penetration into skin & lip tissue causing damage to the elastic fibers and collagen which give lips their characteristic shape, resulting in wrinkling and the appearance of premature aging. Both UVA and UVB radiation have been linked to DNA damage and potentially skin cancer. Therefore, protecting the lips from UVA and UVB radiation is important for the maintenance of lip health and appearance.

The use of significant amounts of sunscreen and often a mixture of organic sunscreens is required to achieve efficacious protection of the lips from both UVA and UVB radiation. Sunscreens, particularly organic sunscreens such as avobenzone have an unpleasant taste. For lotions that are applied to the body this is not an issue, but becomes a significant problem when they are incorporated into lip treatments. Unfortunately, there are no other sunscreens which afford UVA protection as effectively as avobenzone. The resulting challenge was to develop a lip balm stick providing high UVA and UVB protection utilizing taste-masking technology to improve organoleptic properties.

Materials & Methods

The developed formula provides a taste-masked sunscreen composition comprised of multiple sunscreens (including avobenzone), a sorbitol spider ester (sorbeth-2-hexaoleate), and a photostabilizer. The term spider ester is defined as a compound comprised of fatty acid groups esterified to short polyoxyalkylene chains which in turn are attached to a common linking group. The resulting ester has a 3-D molecular structure with limited rotation and a general configuration that resembles a spider. Spider esters have previously been claimed in cosmetic use for moisturization and emolliency for the skin.

Efficient taste-masking of the organic sunscreens with the sorbitol spider ester was achieved when the ratio of total amount of sunscreen to spider ester was approximately 0.6 to 2 by weight. In addition to quantity, it is also believed that the order of mixing of certain components is directly related to the ability to achieve a successfully taste-masked lip treatment composition. The spider ester (sorbeth-2-hexaoleate) was combined with a photostabilizer (diethylhexyl,2-,6-napthalate), and a mixture of sunscreens including homosalate, octylsalate, oxybenzone, octinoxate, and avobenzone prior to combining this mixture with the other formulation components. In this case, use of a photostabilizer is desirable as the formulation includes a sunscreen such as avobenzone, which is susceptible to degradation. Pre-mixing of the spider ester and sunscreen provides for the formation of an intimate association between the 2 components. The term "intimate association" is used as the spider ester and the sunscreen form an interaction or "complex" which is stable at ambient temperatures, but can be separated when heated to a temperature above 100C.

Furthermore, it was determined desirable to warm the spider ester/sunscreen pre-mix to facilitate the formation of the intimate association. Specifically, the premix was heated to about 40C to 80C for 30 minutes to 2 hours with stirring while maintaining the temperature prior to combination with the other components of the lip treatment composition. This premixing, preferably in the presence of warming, is required to achieve taste-masking (i.e. the mere presence of a spider ester and a sunscreen in a composition does not provide taste-masking of the sunscreen).

Results- ***Taste Evaluation***

Compositions containing sorbeth-2-hexaoleate were taste evaluated by a panel of five persons against lip compositions containing an identical sunscreen composition but without the Spider Ester. Both products featured SPF values of 50+ and effective UVA sunscreen activity. The products containing the spider ester displayed excellent taste characteristics, while the product without the spider ester displayed an extremely bad taste. The taste difference between the SPF 50+ (with 3% avobenzone) compositions, with and without the spider ester, was very dramatic and unequivocal. The SPF 50+ (with 3% avobenzone) composition without the spider ester would not be commercially viable because of its very bad taste, nor would this composition be a suitable candidate for additional taste panel testing.

The SPF 50+ (with 3% avobenzone) spider ester composition (Table 1) was subsequently provided to 20 panelists alongside a commercial SPF 30 lip product with no effective UVA sunscreen (without spider ester). All twenty persons classified the SPF 50+ composition with an excellent taste and the SPF 30 commercial product as bad tasting. This result represents a unanimous preference for the more effective sunscreen lip product (SPF 50+ with effective UVA) with spider ester versus the less effective sunscreen product (SPF 30, no UVA).

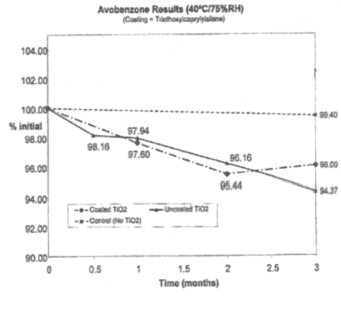
Results - Avobenzone Stability

Titanium dioxide, while classified as a sunscreen is also frequently incorporated into formulations for colorant purposes. Per the sunscreen monograph, avobenzone and titanium dioxide are a permitted combination. Unfortunately, transition metals, like titanium, can promote instability of the Avobenzone. A comparative study of the Avobenzone stability in the taste-masked sunscreen composition was performed at 40C/75%RH to monitor the stability of Avobenzone in a composition that contains coated titanium dioxide versus uncoated titanium dioxide. A composition containing no titanium dioxide served as the study control. The data in Figure 1 demonstrates that the use of coated titanium dioxide retards the degradation of Avobenzone that was observed with uncoated titanium dioxide.

TABLE 1

Ingredient	Amount % wt/wt
Hydrogenated polydecene	4.71%
Paraffin wax	20.00%
Isopropyl myristate	1.00%
Perfluorononyl dimethacone	5.00%
Dimethicone	3.00%
White wax	2.00%
Carnauba wax	1.00%
Methylparaben	0.10%
Propylparaben	0.06%
Cetyl Alcohol	0.50%
Sucralose	0.03%
Preformed Taste-Masked Composition	54.0%
(Diethylhexyl 2,6-Naphthalate (photostabilizer)	0.50%)
(Homosalate.	7.00%)
(Octylsalate	5.00%)
(Oxybenzone	5.00%)
(Octinoxate	7.50%)
(Avobenzone	3.00%)
(Sorbeth-2-hexaoleate (spider ester)	26.10%)
Zinc oxide (50% in suspension)	6.00%
Flavor	1.50%
Vitamin E Acetate	1.00%

FIGURE 1



Summary

Taste is significantly unpleasant to discourage use and/or result in limited compliance of existing lip products with an efficacy of SPF 30 or greater. The addition of avobenzone to provide UVA protection to such compositions would exacerbate the taste problem significantly. The presented formulation resulted in a lip balm stick with high UVA and UVB protection utilizing taste-masking technology to achieve improved organoleptic properties thereby encouraging consumer compliance.

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