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GENETIC RESPONSE OF HUMAN ADIPOCYTES TO EXTERNAL STIMULI: HIGH SPECIFICITY AS REVEALED BY DETAILED DNA ARRAY ANALYSIS AND LIPID STORAGE MODULATION IN A 3D HYPODERMIS MODELE

Karl Lintner[1], Ph.D., Philippe Mondon[1], Ph.D., Nada Andre[1] and Philippe Benech[2], Ph.D.

[1]*Sederma S.A.S., Le Perray en Yvelines, France*
[2]*Predigard, Marseille, France*

Key words: DNA array, sarsasapogenine, glaucine, cell type reversion, data mining

INTRODUCTION:
Adipocytes are more than storage cells. They are highly specialised and possess numerous receptors that interact with their environment. Numerous genes are involved in the regulation of adipocyte physiology.

OBJECTIVE OF THE STUDY:
Various endogenous hormones of different chemical structure (steroids, amines, peptides) modulate adipocyte differentiation, lipogenesis and lipolysis. Surprisingly, non-endogenous substances of plant origin possess similar and contrasting activities. Understanding their specificity and their mechanisms of action would help in the search for further endogenous substances and their regulatory processes.

METHODS:
Murine 3T3L1 and human skin adipocytes were maintained and cultured in appropriate media for 3, 8 and 24 hours; mRNA was extracted in response to stimulation of these adipocytes by the two substances chosen: glaucine (fig. 1) and sarsasapogenin (figure 2) and subjected to DNA chip analysis of whole human genome; to understand the observed gene modulation, data mining software (PredictSearch®) was employed. Fibronectin was detected and quantified by classical fluorescence tagging techniques and subsequent image analysis. Construction of a 3D model of a hypodermis implied embedding adipocyte precursors cells in a dermal matrix composed essentially of collagen proteins; addition of a differentiation cocktail (hormones) to this 3D model led to mature adipocytes that are functional in a three dimensional environment (as opposed to monolayer culture). Image analysis of adipocyte morphology allowed quantification of observed changes.

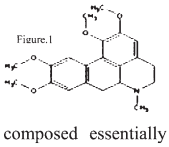


Figure.1

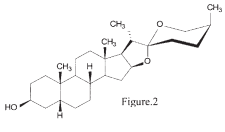


Figure.2

RESULTS:

Out of 43776 gene sequences studied, glaucine stimulated 69 genes significantly (>2fold). Those particularly specific to glaucine are being discussed here. Glaucine is a PDE4 inhibitor (displaces rolipram from its receptor), which leads to increased lipolysis and induced oxidative stress. To protect the cells against this stress, HMOX-1 (heme-oxidase), SLC6A6/TAUT (taurine transport), NUDT4 (elimination of toxic nucleotide derivatives) and SLC22A5/A15 (carnitine transport proteins) are induced. It would thus appear that glaucine, extracted from *Glaucium flavum*, through its ability to enhance intra-adipocyte taurine and carnitine transport and to initiate the cell detoxification process, promotes the triggering of energy production and protection of the most fragile cell fractions or organelles, such as mitochondria.

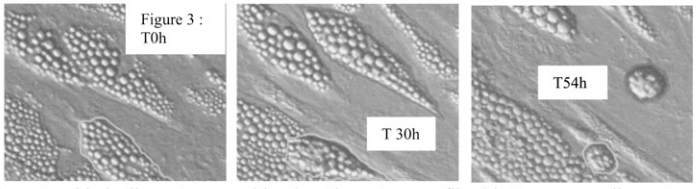
More specifically, however, glaucine participates in the remodeling of the adipose tissue. The induction of TPJ1, a protein implicated in the intercellular tight junctions, activates certain metalloproteinases, of which MMP14 is found to increase significantly. MMP14 is tissue specific, its role being the activation of MMP2 and MMP9 during pre-adipocyte differentiation. It also cleaves integrines and other adhesion molecules [1] and participates in the three dimensional ordering of white adipose tissue [2]. Finally, the gene called ADAM17, coding for a protein known as a "metalloproteinase disintegrin". ADAM17 plays a role in the cleavage and shedding of adipocyte surface proteins, both in the early and mature stages.

The DNA microarray results provide the basis for elucidating the action mechanism with respect to the selected targets:

- Antioxidant effect through reduction of pro-inflammatory cytokine production
- Degradation of fatty acids through activation of carnitine transport
- Adipocyte shedding by proteolysis of intercellular and cell-extracellular matrix junctions
- Remodeling of adipose tissue by 3-dimensional architectural reorganisation

In order to confirm these results, the corresponding *in vitro* studies were performed, which gave the following results: incubation of hypertrophied adipocytes with glaucine leads to significant reduction of IL6 synthesis,

to highly significant increase in lipolysis, and increased ATP production in mitochondria. The remodeling (i.e. shedding of adipocytes) was observed in monolayer culture (under the optical microscope: figure 3) and in reconstructed 3D hypodermis (figure 4: before and after 9 days of incubation). The effect of proteolytic detachment is specific to hypertrophied adipocytes, as neither keratinocytes, nor fibroblasts nor pre-adipocytes are affected.



Fibronectin inhibits adipocyte maturation and lipid storage [3]. This led us to investigate the effect of glaucine on fibronectin production in the 3D model where we find indeed a notable increase of this protein after incubation with glaucine. The tissue remodeling activity of glaucine is thus substantiated and was later confirmed in clinical studies.

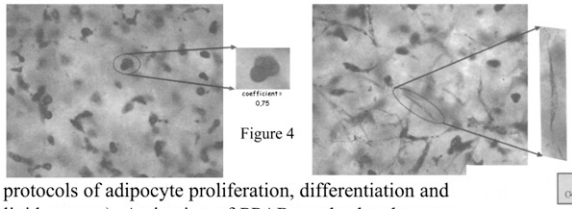


Figure 4

The second plant molecule studied in this context, sarsasapogenine (found in *Smilax aspera* and *Anemarrhena asphodeloides* extracts) had a quite opposite effect on genes (and on the subsequently studied protocols of adipocyte proliferation, differentiation and lipid storage). Activation of PPARγ and related genes such as COPS3 and COPS5 (5 fold increase), Cycline D3 and C/EBPβ all potentiate the differentiation process, allowing pre-adipocytes to become mature, lipid storing adipocytes. Concomitant with this observation is the stimulation of transport proteins (SLC2A5/GLUT5, a solute carrier for fructose and glucose, and RAFTLIN, idem) as well as ADRP (adipophilin, 4 fold) which is involved in the formation of lipid droplets and the formation of long chains of fatty acids. Finally, the installation of new adipose tissue on the matrix is favored by PCOLCE2, LOX and ECM2 proteins. Overall, the set of results paint the picture of a product that activates adipocyte differentiation, stimulates lipid uptake by activation of the glucose/fructose pathway, promotes the formation of lipid vesicles *via* adipophilin and strengthens the installation of fat in the extracellular matrix (figure 5). The clearly opposed activity of this molecule (compared to glaucine) was confirmed in clinical studies (increase of breast volume).

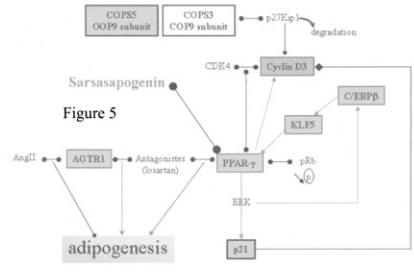


Figure 5

CONCLUSION:

Given the fact that human adipocytes have not necessarily co-evolved with specific non-food plant molecules, it is surprising to see the high specificity of activity of two superficially similar structures on these cells. Although DNA array, data mining, *in vitro* enzymatic and proteomic studies and analysis of 3D hypodermis models allow us to understand in increasing detail the modulation of lipid storage in adipocytes, we still need to find the primary, intrinsic substance regulating these processes. There was no reason to suspect that morphine would bind to pain receptors in the brain until enkephalines were discovered, it can thus be surmised that endogenous molecules exist who are mimicked by the substances tested in our experiments. They would present interesting cosmetic (and pharmacological?) potential.

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NATURAL INGREDIENTS USED AS "PRECURSORS" OF BIOCHEMICAL REACTIONS TO BOOST THE DEFENSE AND REPAIRING MECHANISMS IN THE SKIN: A NOVEL AND SAFE APPROACH TO TREAT ANTI-AGING

Giorgio Dell'Acqua, Ph.D.

R & D Consultant, Induchem AG, Volketswil, 8604, Switzerland

Introduction

One of skin's most important features is to utilize its natural defenses and repair mechanisms to protect and recover from damages caused by the environmental aggressions. However, due to aging, the skin becomes less capable of fully responding to these aggressions due to the fact that many of its mechanisms linked to protection and repair are slowed down or impaired.

Natural ingredients can function as "precursors" of upstream enzymatic reactions to help the skin fight the effects of UV-exposure and aging by reinforcing skin barrier and by accelerating repair of damaged DNA and proteins. We have explored proteins involved in skin barrier differentiation and enzymes responsible for repair after UV damage. In particular, we have performed an extensive investigation using reconstituted human skin and human skin biopsies, to explore the possibility of modulating by using different bioactive complexes: the expression of key proteins necessary for stratum corneum differentiation, the kinetic of repair of UVB-induced DNA damage and the level of oxidized proteins repairing enzymes after solar UV exposure. We have also investigated some clinical correlation in healthy volunteers.

Methods

Compounds

Bioactive complex 1: hydrolyzed oat protein, niacinamide, ATP
Bioactive complex 2: panthenyl triacetate, ethyl linoleate
Bioactive complex 3: acetyl tyrosine, proline, hydrolyzed vegetable protein, ATP

In vitro Studies

Skin Models
Reconstituted human skin (SkinEthic®, France, 0.50 cm^2, 17 days old) and human skin biopsies.

UV-irradiation and treatment
UVB irradiation (300 mJ/cm^2) on reconstituted human skin and UVA+B irradiation (1.5 J/cm^2 UVB and 20 J/cm^2 UVA) on human skin biopsies were performed. Before and after UVB irradiation the skins were treated with 1% or 3% Bioactive Complex 3 and left for 5 hours. Before and after UVA+B irradiation the skin samples were treated with 3% Bioactive complex 2 and left for 24 hours.

DNA Chip Analysis
mRNA was extracted from reconstituted human skin treated and untreated for 24 hours with Bioactive complex 1. Gene expression analysis was performed using standard minichips (BIOalternatives, France) consisted in 164 target genes representative of keratinocytes' physiology. The cDNA ^{33}P-labelled targets were prepared by direct reverse-transcription of the extracted mRNA. These targets were hybridized to the specific cDNA probes covalently fixed to the minichips. Hybridization was revealed by Phosphor Imaging.

RT-PCR
mRNA was extracted from reconstituted human skin or human skin biopsies treated and untreated for 24 hours with Bioactive complex 1 or Bioactive complex 2. Reverse transcription of mRNA was conducted in presence of oligo(dT) and Superscript II reverse-transcriptase (Invitrogen). The PCR (Polymerase Chain Reaction) was performed in triplicate using the « LightCycler® » system (Roche Molecular Systems Inc.). For differentiation markers: Filaggrin (FLG), Small proline-rich proteins 1B-2A (SPR1B-2A) and Late envelope protein (LEP) were amplified. For protein repairing enzymes: Glutathione Reductase (GSR), Methionine Sulfoxide Reductase A and B2 (MSRA/B), and Thioredoxin (TXN) were amplified.

Cyclobutane pyrimidine dimer (CPD) detection and quantification
Reconstituted human skin sections were incubated with the primary monoclonal antibody (anti-thymine dimer, Kamiya Biomedical Company, Seattle, USA) at 4°C overnight. After washing sections were treated with a signal enhancer polymer labeled with alkaline phosphatase (AP) molecules (DAKO®Diagnostika GmbH) conjugated to goat anti mouse and anti rabbit IgG for 1 hour at RT. Visualization of the CPD´s was performed by incubating the sections in a substrate/chromogen solution (DAKO® Fuchsin Substrate/ Chromogen system) for 8 min. Quantification was obtained scoring CPD according to number and intensity.

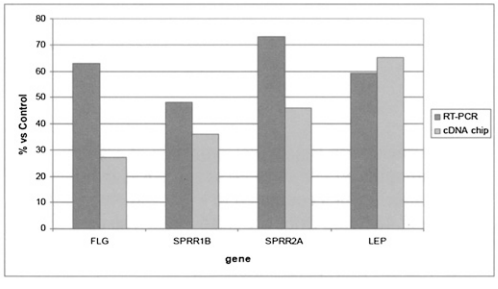
In vivo Studies (Human Volunteers)

Experimental Group
Twenty-five volunteers (males + females) were selected from the Derma Consult Concept GmbH database (Dusseldorf, Germany). They were informed about the importance and concept of the study. Written informed consents were obtained from all the subjects prior to entry into the trial. The following criteria were used for subject inclusion in the study: older than 18 years of age, clinically healthy; and for exclusion from study: skin diseases, pregnancy, uneven skin tone, sunburn, scars or lesions in the test area, skin phototype (Fitzpatrick) > III.

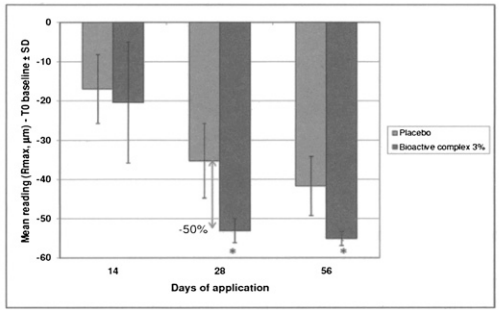
Skin Redness Induction
Skin redness was measured after pre-treatment with a placebo cream or a cream containing 3% Bioactive Complex 3, followed by UV irradiation. Tested areas were irradiated with UV light (Solar Light Model 601-300 Multiport) adjusted to have a 1.25 x MED. Skin redness (representing erythema) was measured 24 and 48 hours after UV irradiation with a Chromameter CR 400 (Minolta, Japan).

Skin Wrinkles Depth Measurement
Crow's feet around the eye area were measured for their depth. One side of the face was treated with a cream containing Bioactive Complex 1 at 3% and the other side with a placebo cream. Further measuring was performed after 14, 28, and 56 days of application. The crow's feet areas were recorded as a 3D topography using the PRIMOS system by means of the parameter RMax that is defined as the maximum vertical distance from the highest peak to the lowest valley of five segments of equal length.

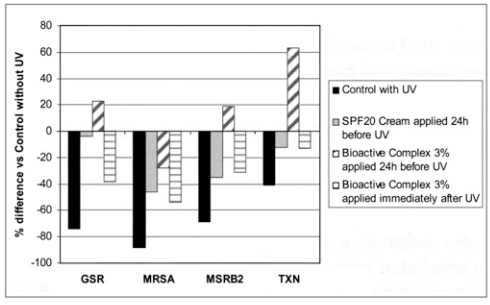
Results



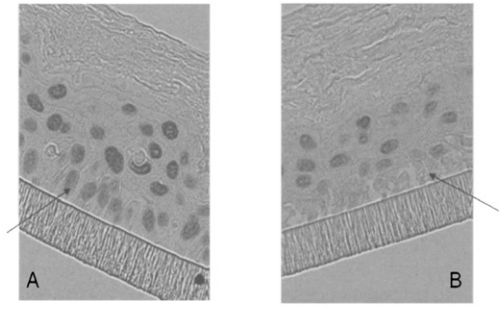
cDNA microchip and RT-PCR analysis show that Bioactive Complex 1 at 1% increased by more than 40% the mRNA of filaggrin, small proline-rich proteins and late envelope protein when compared to untreated control in reconstituted human skin. FLG: filaggrin; SPRR1B-2A: small proline-rich proteins 1B and 2A; LEP: late envelope protein



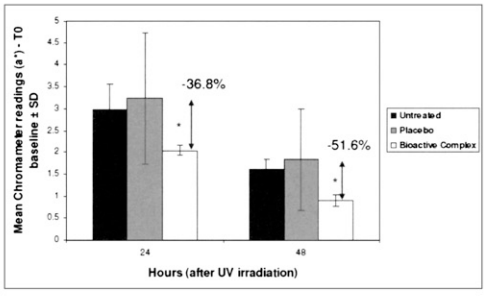
Bioactive Complex 1 at 3% in a cream decreased wrinkle's depth in 25 human volunteers, when compared to a placebo cream. 50 % decrease was achieved after 28 days of treatment. Data were statistically significant (*$p < 0.01$ vs Placebo, Student's T test).



Pre- and Post- treatment with Bioactive Complex 2 at 3% stimulated protein repairing enzymes that were down-regulated by UV irradiation in human skin biopsies. Data are expressed as % of non irradiated control. Reference compound was an SPF20 cream. GSR: Glutathione Reductase; MSRA/B2: Methionine Sulfoxide Reductase A/B2; TXN: Thioredoxin.



Treatment with Bioactive Complex 3 at 3% followed by UVB irradiation accelerated cyclobutane pyrimidine dimer (CPD) removal when compared to control. Red staining indicates CPD positive nuclei. As it can be observed in picture B, number of positive nuclei and intensity were strongly reduced (see basal layer, arrows), when compared to irradiated control at same time (A)



Treatment with Bioactive Complex 3 at 3% in a cream reduced UV-induced skin redness in human volunteers when compared to a placebo cream after 24 and 48 hours from UV irradiation. The data were statistically significant (*$p < 0.01$, Student's T test). a*= green-red, redness indicator

Discussion and Conclusion

In this paper we have demonstrated how mixtures of natural precursors such as pro-vitamins (Panthenyl Triacetate, Niacinamide), amino acids (Acetyl Tyrosine, Proline), energy boosters (ATP) and protein hydrolizates can indeed trigger physiological mechanisms associated to clinical effects. We can speculate that, in our experimental settings, pro-vitamins together with ATP could boost metabolic reaction associated to protein or lipid synthesis, while amino acids such as acetyl tyrosine and proline may either function as a resource pool for protein kinases assembly or provide an activity on their own either as antioxidants or survival factors as suggested by the scientific literature. Finally protein hydrolisate rich in glutamine could function as substrate for glutamate synthesis followed by activation of differentiation.

These activities result in increasing the expression of necessary enzymes and structural proteins needed to provide adequate protection against environmental challenge such as UV. Current anti-aging strategy provides functional molecules such as downstream actives (vitamin A, C, peptides, anti-oxidants, etc.), but although very efficient still runs into "overdosing" and safety issues associated with skin irritation, often limiting the use to individuals not having sensitive skin. We believe this approach to anti-aging that provides pro-active precursors that would be integrated or transformed into active molecules only when needed by aged or UV-stressed skin, is equally effective without the potential of sensitization and with a better tolerance by sensitive skin.

CONFOCAL RAMAN STUDIES OF STRATUM CORNEUM BIOCHEMISTRY AS A FUNCTION OF CLIMATE

David Koenig[1], Ph.D., Andrew Basehoar[1], Andrea Smiltneek[1], Douglas Hoffman[1], Barry Reece[2] and Corey Cunningham[1], Ph.D.

[1]*Kimberly-Clark Corporation, Neenah, WI*
[2]*Reliance Clinical Testing Services, Irving, TX*

Abstract
Relatively little has been done to investigate the impact of season and/or climate on skin biochemistry. The objective of this work was to investigate differences in skin biochemistry across two climates using *in vivo* Raman confocal spectroscopy. The study spanned summer to winter in Neenah, WI and Dallas, TX. Daily temperature and dew point were recorded for both locations. Skin analyses included TransEpidermal Water Loss (TEWL), impedance, and Raman spectroscopy for biochemical components. The natural moisturizing factors urocanic acid and lactate were the only components to differ with climate conditions. They were found to be significantly lower during hotter/humid conditions. The lactate component was significantly associated with ambient temperature at deeper (>20 μm) skin depths while urocanic acid was more strongly associated with temperature at the skin surface. Observations from these studies suggest climate is unlikely to be an important covariate in Raman analyses for all components other than urocanic acid and lactate.

Introduction
The skin is the body's largest organ is constantly exposed to environmental conditions. It is well known that conditions such as temperature and relative humidity can have a significant impact on skin features, including barrier function and moisturization. For example, it has been reported that changes in the concentration of free amino acids in the stratum corneum can change as a function of humidity[1]. The skin has also been shown to be more susceptible to irritant damage in the winter than in the summer[2]. These and other reports underscore the importance of considering climate and environment in all skin-related studies. *In vivo* confocal Raman microspectroscopy is a non-invasive technique capable of measuring biochemical components in skin, such as lipids, amino acids, and water. A recent study using an Asian-descent subject group (n=44) observed differences in lactate, urea, and *trans*-urocanic acid as a function of season[3]. An opportunity exists to further the understanding of the impact of climate on Raman measurements for skin using a more diverse and larger sample group.

Materials and Methods
A total of 87 subjects (77 female, 10 male) of different age groups, race, and skin type participated in this study. Fifteen (15) subjects were <3 years old, 50 were 25-35 years old, and 22 were 65+ years old. Forty-three (43) subjects were Caucasian, 17 African-American, 21 Hispanic, and 6 Asian/Pacific Islander. The study spanned from summer to winter in two locations (Neenah, WI and Dallas, TX). Temperature and relative humidity was recorded at each location on each day of testing. Prior to evaluations all subjects equilibrated for at least 15 min in an environmentally controlled room. Following equilibration, Raman measurements (Model 3510 Skin Analyzer, Rivers Diagnostics®, Rotterdam, The Netherlands) were completed on the volar aspect of the forearm. A minimum of seven (7) scans were completed per site per subject for both water content (HWN) and skin lipids/Natural Moisturizing Factor (NMF). Data was processed using methods previously described[4]. The relative concentration of individual components was compared as a function of average temperature and relative humidity at each location at the time of measurement.

Table 1: Mean temperature and relative humidity during each study period.

Location	Timing	Temperature (°C)		Relative Humidity (%)	
		Mean	SD	Mean	SD
Neenah, WI	Summer	22.8	5.9	71.1	7.3
Neenah, WI	Fall/Winter	2.3	13.1	75.5	12.2
Dallas, TX	Summer	27.5	3.9	66.7	9.3
Dallas, TX*	Fall/Winter	10.3	12.1	61.6	18.2

SD=standard deviation
*Only water content (HWN) was measured in this study. No NMF measurements were made.

Results and Discussion

Comparisons for total NMF, water (HWN), ceramide 3, cholesterol, urea, and *trans*-Urocanic acid (pH 8) as a function of temperature and relative humidity showed essentially no change down to a depth of 24 µm (data for water shown in Figure 1 as an example). Egawa and Tagami[3] reported a seasonal change in urea, lactate, and *trans*-urocanic acid (pH 4) levels near the skin surface. In this study we observed a seasonal change for lactate and *trans*-urocanic acid (pH 4). In contrast to Egawa and Tagami[3], we found lactate to be more prevalent during the fall/winter period than the summer (Figure 2A). This change was noted deeper in the skin (24 µm) rather than at the skin surface. Similar to Egawa and Tagami[3], we observed *trans*-urocanic acid (pH 4) to be more prevalent during the fall/winter period (Figure 2B). It is hypothesized that the shift in seasonal prevalence of these components is related to adjustments made within the skin to maintain homeostatic water levels during changing climate conditions.

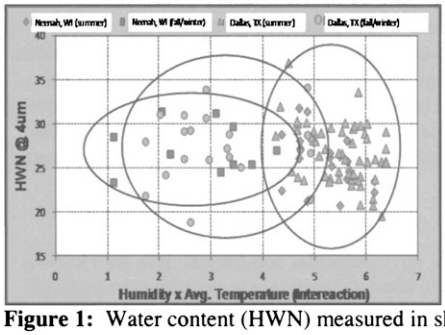


Figure 1: Water content (HWN) measured in skin as a function of temperature and relative humidity.

These results imply climate and change in season can impact Raman microspectroscopy measurements though only for a relatively small portion of the measured components. Future studies examining lactate and *trans*-urocanic acid (pH 4) levels in skin should factor potential seasonal fluctuations into their experimental designs and data analyses.

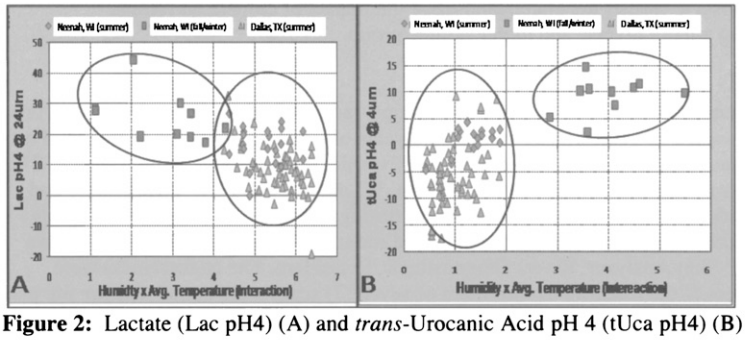


Figure 2: Lactate (Lac pH4) (A) and *trans*-Urocanic Acid pH 4 (tUca pH4) (B) measured in skin as a function of temperature and relative humidity.

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THE LIGHT ON KEY REGULATOR PROTEINS IN CELLULAR MITOSIS AND ADULT EPIDERMAL STEM CELL DIFFERENTIATION

Isabelle Imbert, Ph.D., Florian Labarrade, Celine Meyrignac, Alexia Lebleu, Jean-Marie Botto, Ph.D., Catherine Gondran, Ph.D., Claude Del Farra, Ph.D. and Nouha Domloge, MD

ISP-Vincience, 655 Route du Pin Montard, Les Bouillides, BP 212, Sophia-Antipolis, 06904, France

INTRODUCTION:

The epidermis and the hair follicle need to continuously generate new cells through proliferation of progenitor cells and subsequent differentiation. This renewal is permitted by adult stem cells (ASCs) originating from the bulge region of follicles [1, 2]. Maintenance, proliferation or differentiation of ASCs seem to be tightly modulated by changes in genes expression such as the Chromosomal Passenger Complex (CPC) components (survivin, borealin, incenp, aurora kinase B) and p63 TA and ΔN variants (TAα, β, γ and ΔNα, β, γ) [3, 4, 5]. The objective of this study was to better understand the role of these key regulators in the correct development of cellular mitosis, essential for adult skin stem cell maintenance and protection.

MATERIAL AND METHODS:

Fractions of primary cells from human epidermis were prepared based on adhesion time to collagen IV [6]. RT-PCR and immunoblotting were performed to quantify expression of the CPC components and p63 variants at the genomic and proteomic level.

To investigate interactions between different CPC components, the four CPC genes were separately silenced by a specific siRNA in HaCaT cells.

RESULTS:

Fractionation of primary keratinocytes, based on adhesion time to collagen IV allowed selection of several enriched population of cells: 20 minutes for rapid adherent (RA), 5 hours for slowly adherent 1 (SA1), 16 hours for slowly adherent 2 (SA2) and finally non adherent keratinocytes (NA) (Figure 1).

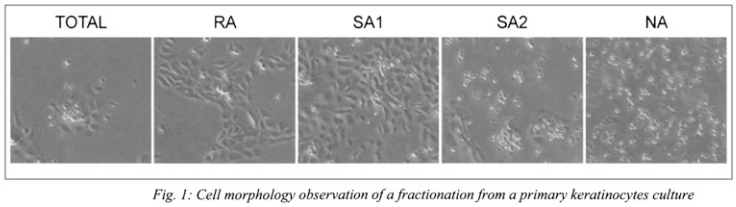

Fig. 1: Cell morphology observation of a fractionation from a primary keratinocytes culture

RT-PCR experiments showed that p63 TA isoforms were mainly expressed in cells populations which were enriched in progenitor cells. On the contrary, the TA isoforms were barely detectable in differentiated cells. This indicates a switch in the expression of p63 TA isoforms during keratinocytes differentiation.

RT-PCR analysis of the CPC members in keratinocytes fractions showed a different pattern of mRNA expression. In NA cells, only borealin transcript was detected (Figure 2).

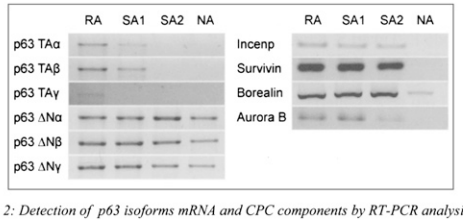

Fig. 2: Detection of p63 isoforms mRNA and CPC components by RT-PCR analysis

Immunoblotting assay revealed that survivin was naturally expressed at a higher level in RA cells and decrease progressively in SA1, SA2 for finally not being expressed in NA cells (Figure 3).

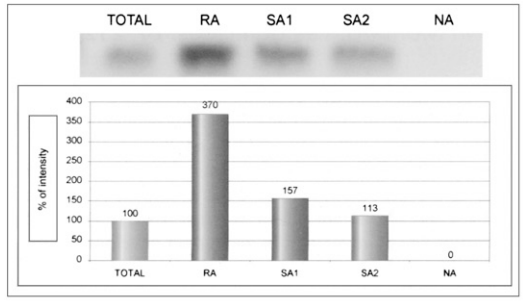

Fig. 3: Survivin immunoblotting in keratinocytes subpopulations

To investigate interactions between different CPC components, the expression of the four CPC genes were separately silenced by a specific siRNA in HaCaT cells.

Results indicated that a depletion of one member of the CPC had a significant impact on the other 3 CPC proteins (Figure 4).

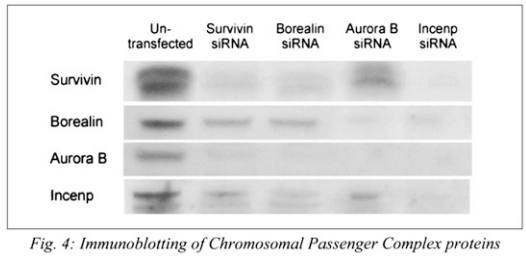

Fig. 4: Immunoblotting of Chromosomal Passenger Complex proteins

CONCLUSION:

These studies shed more light on epidermal adult stem cell physiology and the crucial role of Chromosome Passenger Complex proteins. It appears that components of the CPC are tightly regulated by feedback loops to control the correct development of cellular mitosis.

All together, these new results revealed the importance of survivin and p63 TA isoforms expression in the maintenance of progenitor cells. These new data should be of particular interest to investigate future development of biofunctionals targeting adult epidermal stem cells protection.

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Tight Junctions and Flow of Lipids: New Cosmetic Targets for Restoring the Skin Barrier Function

David Boudier, Sylvain Mazalrey, Sandrine Gofflo and Brigitte Closs

R&D Department, Silab, France

Introduction

The main function of the skin is to protect the body against exogenous substances and excessive water loss. The skin barrier is located in the outermost layer of the skin, the *stratum corneum*. This barrier consists of protein-enriched cells (corneocytes with cornified envelope and cytoskeletal elements, as well as corneodesmosomes) and lipid-enriched intercellular domains.

Recent investigations for understanding in depth the homeostasis of the skin barrier function have permitted to highlight new cell signaling pathways: the dynamic flow of lipids and the tight junctions (**Figure 1**).

Indeed, the process of synthesis, transport, secretion and maturation of epidermal lipids is a dynamic equilibrium ensuring homeostasis of the barrier function. The production of lamellar bodies, which release their lipid contents into the intracellular spaces, continuously regenerates the lipid cement of the skin barrier and also responds to alterations that might damage it.

This dynamic process has several steps. The lipids are first synthesized by the epidermal cells in the form of polar precursors—glucosylceramides, cholesterol and phospholipids—from metabolic intermediates and fatty acids. The fatty acids may come from extra-cutaneous sites, and are internalized by the keratinocytes by means of specialized membrane transporters such as the fatty acid transporter proteins (FATP).

These lipid precursors are generated in sufficient quantities and stored in small secretory organelles formed from the Golgi apparatus and known as lamellar bodies (or Odland bodies). The lamellar bodies appear in the upper layers of the spiny layer and increase in volume and size in the granular layer. Inclusion of the lipids within the lamellar bodies is ensured by proteins located on their membranes, the ABCA12s. In addition to the lipid precursors, the lamellar bodies also contain lipases for lipid maturation, proteases and protease inhibitors regulating desquamation, structural components of the corneal envelope and anti-microbial peptides [**1**].

The lamellar bodies fuse with the apical part of the plasma membrane of a keratinocyte in the terminal differentiation phase and secrete their lipid contents into the intercellular space at the junction between the *stratum granulosum* and the *stratum corneum*. The secreted lipid precursors undergo modification through the action of specific enzymes (β-glucocerebrosidase, phospholipases and sphingomyelinase) released at the same time into the intercellular spaces. The modification of the polarity and the structure of the precursors results in the formation of lamellar membranes, the composition and arrangement of which determine the functional characteristics of the barrier function. A particular class of ceramides, the omega-hydroxyceramides, are bound covalently to the cornified envelope of the corneocytes. They anchor the corneocytes to the lamellar membranes and structure the membranes [**2**].

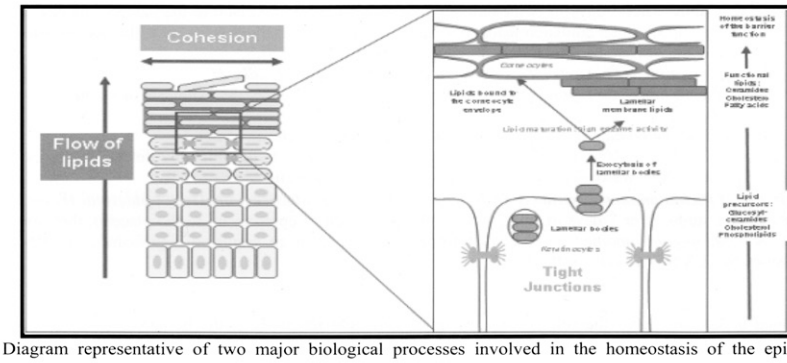

Figure 1: Diagram representative of two major biological processes involved in the homeostasis of the epidermal barrier function.

Regarding the tight junctions, it has recently been accepted that, in addition to the *stratum corneum*, intercellular junctions – and, especially, tight junctions – also play a role in homeostasis of the skin barrier function [**3**; **4**].

Tight or impermeable junctions are, in fact, specialized intercellular junctional complexes that ensure adhesion between the keratinocytes of the *stratum granulosum*. By sealing the plasma membranes of two adjacent cells, the tight junctions form selective semi-permeable barrier preventing water and solutions from "leaking" out towards the surface of the skin via the paracellular space. Tight junctions therefore help maintain moisturization of the various layers of the epidermis [**5**].

Cohesion of the various epidermal layers – and particularly that of the *stratum granulosum* and the *stratum corneum* – is crucial for ensuring homeostasis of the barrier function and limiting excessive water loss.

Consequently, we developed two natural active ingredients capable of stimulating these two very recent cell signaling pathways in order to restore the skin barrier function, one of the main concerns in cosmetic.

Material and methods:

Study of expression of FATP-3, glucosylceramide synthase and ABCA-12 by Q-PCR:
Normal Human Keratinocytes (NHK) were treated with the *Pichia anomala* extract at 0.15% and 0.30% (V/V). After treatment, cells were recovered and total RNA was extracted and analyzed by Q-PCR.
Study of the activity of β-glucocerebrosidase:
The study was conducted on 20 healthy female volunteers of mean age 31±7 years and with normal skin on the arms. Samples of *stratum corneum* were taken with adhesive tape (D-Squames®, CuDerm) before and after 7 days of twice daily applications of the *Pichia anomala* extract formulated at 3% and in comparison to a placebo following a chronic artificial disruption of the barrier function by sodium lauryl sulfate (SLS), to induce the disorganization of the lipid structure of the *stratum corneum*. The activity of β-glucocerebrosidase was assayed according to the modified method of Redoules et al and Takagi et al. [**6**; **7**]
Study of ZO-1 and Claudin-1 by Western Blot:
NHK were treated with the *Ophiopogon japonicus* root extract at 0.50% and 1% (V/V). After treatment, cell proteins extracts were recovered and analyzed by Western Blot.
Study of ZO-1 by immunocytology:
NHK were treated with a solution of 1 mM CaCl2 for 48 h to enable the formation of the tight junction network then were aggressed with Sodium Lauryl Sulfate 0,4 mM for 30 minutes. At the end of incubation, the SLS solution was eliminated and replaced by medium containing 1% (V/V) of the *Ophiopogon japonicus* root extract. After incubation for 48h, cells were fixed and stained with a primary antibody anti-ZO-1 and a secondary antibody Alexa Fluor-488. Visualization was with an IX 70 microscope (Olympus, Japan) coupled to an image analysis system (NIS-Elements software, AR-NIKON).
Effect of the mixture of Pichia anomala extract & Ophiopogon japonicus root extract on TransEpidermal Water Loss (TEWL):
The study was conducted on 18 healthy female volunteers of mean age 31± 4 years with normal skin on the arms. Measurements were made with a TM 210 Tewameter® (Courage & Khazaka, Germany) after 7 days of twice daily applications of the mixture *Pichia anomala* extract + *Ophiopogon japonicus* root extract formulated at 3% and in comparison to a placebo, following a chronic artificial disruption of the barrier function by sodium lauryl sulfate (SLS), to induce the disorganization of the lipid structure of the stratum corneum.

Results:

Effect of the Pichia anomala extract on the expression of FATP-3, glucosylceramide synthase and ABCA-12:
In NHK, the *Pichia anomala* extract increases significantly the expression of FATP-3 by 19% at 0.15% and by 33% at 0.3%, the expression of glucosylceramide synthase by 8% at 0.15% and by 22% at 0.30% and the expression of ABCA-12 by 7% at 0.15% and by 25% at 0.30%.
Effect of the Pichia anomala extract on the activity of β-glucocerebrosidase:
In the conditions of this study, after 7 days of twice daily applications and in comparison to the placebo, mannans of the *Pichia anomala* extract formulated at 3% in an emulsion leads to a significant increase in the activity of β-glucocerebrosidase of 38% (P = 0.0987) after a chronic disruption of the lipid barrier by repeated applications of SLS.
Effect of the Ophiopogon japonicus root extract on the synthesis of ZO-1 and Claudin-1:
Tested at 1% in NHK, the *Ophiopogon japonicus* root extract significantly increases the synthesis of claudin-1 by 26% and the synthesis of ZO-1 by 27%.
Visualisation of the effect of the Ophiopogon japonicus root extract on the synthesis of ZO-1:
After SLS aggression of NHK, the ZO-1 membrane network induced by CaCl2 is considerably deteriorated. Tested at 1%, the *Ophiopogon japonicus* root extract restores the formation of the ZO-1 membrane network of NHK.
Effect of the mixture of Pichia anomala extract & Ophiopogon japonicus root extract on TransEpidermal Water Loss (TEWL):
In the conditions of this study, after 7 days of twice daily applications and in comparison to the placebo, the mixture of *Pichia anomala* extract & *Ophiopogon japonicus* root extract formulated at 3% in an emulsion significantly reduces TEWL after repeated aggressions by SLS by 9.7% (P = 0,0181)

Conclusion
More sophisticated understanding of the role of flow of lipids and tight junctions in the homeostasis of the barrier function enabled us to develop two natural active ingredients capable of stimulating these two indispensable cell signaling pathways. This represents a natural response to skin disorders due to impaired skin barrier function and is recommended for all skin care products claiming a restoring / moisturizing effect.

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KEYNOTE AWARD LECTURE SPONSORED BY RUGER CHEMICAL CORPORATION

A REVIEW OF THE PROGRESS OF FOAMING CLEANSING PRODUCTS DURING THE LAST FIFTY YEARS

Ricardo Diez, Ph.D.

Chanel, Inc., R & D, 876 Centennial Ave, Piscataway, NJ, 08854

This review presents some of the key improvements achieved in the foaming cleansers category since the IFSCC was founded in 1959. The improvements are shown in terms of new "product generations". The sophisticated products of today are the "fifth generation".

The fundamentals of current personal care cleansers go back to the 1920's. At that time, brilliant German chemists were synthesizing new chemicals to overcome the performance weaknesses of soap in the textile industry. The new chemicals, first named "soap alternatives" or "detergents", are the surfactants of today. The patents behind widely used anionic surfactants (sulfates, isethionates, taurates, phospates, etc), nonionics (and roots of amphoterics) were filed in Germany from 1928 to 1935. (1)-(8)

The benefits of replacing soap in personal cleaners with the new surfactants were soon recognized. In 1934 P&G introduced Drene, the first shampoo based on surfactants. The benefits of surfactants were obvious, but their complex manufacturing and higher cost limited their broad use until the end of World War II.

Second Generation

The foundation of the IFSCC in 1959 coincides with the expansion of cleansers made with surfactants, which offered clear benefits relative to soap-based products (our First Generation). It should be emphasized that the extended use of surfactants that started in the 1940's was made possible by advances, often unreported, in chemistry, chemical engineering, and manufacturing equipment.

Surfactants completely transformed the category, improving existing cleansers, creating new ones and, very important, making them widely accessible. Surfactants changed forever hair shampooing, and created the body washes, shower gels and hand cleansers so ubiquitous today. In the bar category, they created the beauty bar. In 1955 Lever introduced Dove. J. Bodman and R. Geitz patents are behind the product (9)(10).

The Second Generation of cleansers are the products, in bar or liquid forms, which offer significant improvements in mildness, lathering, cleansing and rinsing, along with convenience, and affordable cost.

Third Generation

In 1947, H .Manheimer filed the first of several patents about surfactants of rather complex structure (11). However, it was not until 1955 when he disclosed in new patents (12) (13) that shampoos made combining his surfactants (known today as amphoterics) with the commonly used anionic sulfates were "*substantially non-irritating to the skin and eyes of normal human beings*". The concept of mildness, and the chemistry to deliver it, was pioneered in those patents. Soon, the "No More Tears" Baby Shampoo was marketed by J&J. The patents by Masci et al. and Verdichhio et al. improved mildness to the no-irritancy level (14)-(16).

The mildness benefit, however, remained limited to few products until the 1980's, when mildness (not necessarily at the no-irritation level) became a requirement. The new products intended for daily use needed to be mild. From that moment on, the anionic-amphoteric surfactant combination became very popular. It remains the standard system for most personal care products of today.

Products with improved mildness, up to the level of non-irritation to eye and skin, are the Third Generation.

Forth Generation

The excellent cleansing properties of surfactants may result sometimes in hair and skin feeling dry. The idea of adding agents to leave the skin or hair conditioned occurred soon, with shampoos taking the lead.

In 1958 H. Geen patented the first "Non-Tangling Shampoo", containing a silicone as conditioner (17). The execution of the idea in a satisfactory product was a challenge, but in the 1980's, P&G introduced Pert, the first "2 in 1"shampoo. Fieler et al. (18) describe well the challenges of these products.

The second approach to obtain conditioning benefits has relied on adding cationic compounds, an idea presented already in a 1954 patent by Anderson (19). The real break-trough occurred with the development of new cationics, the poly-quaternaries, such as those disclosed in the1965 patent by Stone et al. (20).

The benefits of adding poly-quats to cleansers made with surfactants were described by J. Parran in 1967 as "*improved capacities to impart residual activity or properties to surfaces washed*". (21) The "*residual activity*" that poliquats exert on hair and skin (via the "Lockhead Effect" or coacervate formation) in terms of conditioning is such that practically all of today's shampoos and showers gels are formulated with them.

The Fourth Generation corresponds to cleansing products that deliver conditioning benefits to hair or skin by means of controlled deposition of the desired agents.

Fifth Generation

"It is generally recognized by both laymen and dermatologists that ...skin cleansing preparations....may remove oils from the skin during use...and that to prevent skin dryness...lotions and cream .are widely used, particularly among women". Why not just put the cleanser and cream, together? Barry et al. did that in 1974 (22) combining surfactants with petrolatum and lanoline, and pioneering the Fifth Generation.

Combining "a cleanser and a cream" into one single product that delivers both good cleansing and a measurable hydrating benefit is difficult. The 1997 patent by S. Puvvada (23) is a good example of the improvements made since 1974. The patent also shows how the chemistry improvements previously discussed have been used to achieve these superior products.

These advanced hydrating cleansers are based on surfactants (Second Generation), and are mild (Third Generation) due to the right combination of anionic-amphoteric. The conditioning benefits (Fourth Generation) are provided by a polyquat that also helps the deposition of enough emollient on the skin to provide measurable skin hydration. Product stability is delivered by means of "structured surfactants".

The Fifth Generation products deliver cleansing, mildness, conditioning and real hydration to the skin.

Specialty and Facial Cleansers

This review has focused on the improvements of the cleansers used everyday by everybody, "the family cleansers". There are also the "specialty" cleansers that have created market niches delivering benefits, such as antibacterial, acne or dandruff control, which in some countries are considered drug claims.

In recent years, facial cleansers have created their own new category with unique characteristics. The face is very sensitive because of a thin stratum cornea. Therefore, facial cleansers need to be not only mild but must also convey mildness and softens. Hydration is also a desirable benefit. These attributes of mildness, conditioning and hydration make them part of the Fifth Generation

The higher cost commanded by facial cleansers has opened the door to new formulations and other raw materials. The very creative approaches of some products may contain, and probably already do, the roots of the next product generations. Time, however, is always required to judge a real break-through.

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HYPERBRANCHED POLYALPHAOLEFINS IMPROVE SHINE, DURABILITY AND STABILITY OF COLOR COSMETIC FORMULATIONS

Melanie Urdiales

New Phase Technologies, Sugar Land, TX

INTRODUCTION

Film-forming properties and crystal modification capabilities of hyperbranched polyalphaolefins (HBPs) in color cosmetic applications were studied. In this study, four HBPs ranging from solids with long branch lengths (HBP #1) to liquids with short branch lengths (HBP #4) were studied. Due to their high refractive index (1.47-1.51) and molecular structure, these polymers offer an excellent alternative to traditional ingredients in providing gloss, durability, and stability to color cosmetic formulations.

METHODS

Gloss measurements were conducted using a HunterLab gloss meter following a standard method for specular gloss [1]. Using a circular motion for one to two minutes, specimen of each test formula, weighing 0.5g, was applied to a 4.5 x 5.5-inch test surface; gloss readings were taken at an angle of sixty degrees. Scanning Electron Microscopy (SEM), pour point temperature, and hardness measurements were utilized to determine the extent of crystal modification. Pour point temperatures were recorded as the lowest temperature at which the mixture was still fluid and pourable. Hardness measurements were recorded after conducting a standard procedure [2] using a Koehler penetrometer with a 35g cone needle. Transfer resistance lipstick formulas were evaluated for wear and aesthetics by a twelve-member panel.

RESULTS & DISCUSSION

Traditional Lipstick Formula

Hyperbranched polyalphaolefins (HBPs #1-4) *(INCI name synthetic wax)* were evaluated for gloss, durability, and stability in traditional lipstick formulas. Simple base formulas consisting of polyethylene in five cosmetic oils were studied. The oils evaluated were mineral oil, safflower oil, isododecane, isopropyl palmitate, and C_{12}-C_{15} alkyl benzoate. The HBPs were added at five percent into these base formulas. Common glossing agents, phenyl trimethicone and hydrogenated polyisobutene, were also included as benchmark materials. Given the HBP's high refractive index (1.47-1.51) and their film-forming capabilities, they were able to provide significantly higher gloss over the base formulas and benchmark materials (Fig 1) [3]. The HBP's film-forming capabilities are attributed to their unique molecular structure and their ability to modify the crystal lattice of crystalline waxes or polymers as shown in SEM photos (Fig 2).

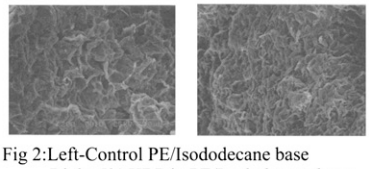


Fig 2:Left-Control PE/Isododecane base
Right-5% HBP in PE/Isododecane base

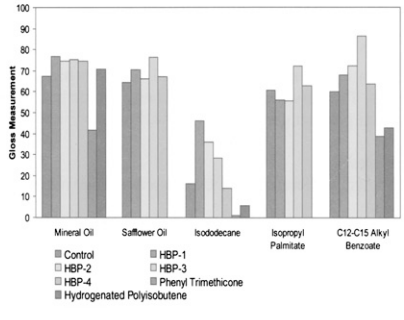
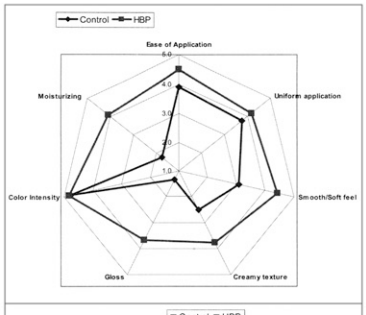


Fig 1: Gloss of base formulas with 5% HBPs

In this study, modifying the crystallinity of polyethylene provided other unique benefits such as decreased pour point temperatures and improved syneresis. When the HBPs were added to the base formulas, significant reductions in pour temperatures were recorded. The use of HBPs as pour point depressants provides the formulator the added benefit of handling volatile or temperature-sensitive ingredients with greater ease.

Transfer-Resistant Formula

Transfer-resistant lipstick formulas containing HBPs were evaluated by a twelve-member panel for aesthetics, gloss, and long-wear properties. The formula contained isododecane with polyethylene as the main structurant and an acrylate copolymer as the non-transfer film former. Panel results showed that the panelists consistently rated the formula containing HBPs higher for initial gloss and uniform application. Aesthetics, such as moisturizing feel, lipstick payout, and creamy/smooth texture, were also improved over the control (Fig 3). For long-wear properties, panelists applied the lipstick in the morning and ranked wear properties at several hour intervals throughout the day. Although an acrylate copolymer was used as the transfer-resistant film former, the addition of the HBPs improved the long-wear properties of the formulation (Fig 4). Lab tests indicated that the HBP formula had improved anti-flake properties. These results led to the hypothesis that by modifying the crystallinity of the polyethylene film, flexibility can be improved. Improved film flexibility may lead to improved wear properties. Stability studies were conducted at room temperature and at 49°C for six weeks. Elevated temperature results indicated the formula containing a medium branch length HBP provided better stability, and reduced shrinkage versus the control.



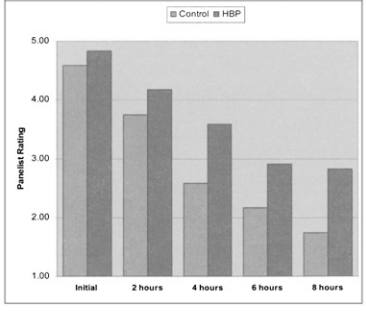


Fig 3 (top): Panelists Initial Rating *(5=best 1=worst)*

Fig 4 (bottom): Long Wear Rating *(5=long wear1=no wear)*

HBPs as Pigment Dispersants

Pigment grinds of non-functional HBPs and alcohol functional HBPs were evaluated in combination with organic lakes. Improved color development and compatibility versus controls were observed. Initial observations indicated that the alcohol functional HBP provided improved pigment dispersion and compatibility within polar oils. At this point more work is being conducted to investigate optimizing the amount of functionality of the new functional HBPs.

CONCLUSION

By incorporating the HBP's unique molecular structure into color cosmetic formulas, the formulator has the ability to modify polymer crystallinity. Crystal lattice modifications were shown to lower pour point temperatures, increase gloss and improve film flexibility. The added possibility of alcohol functional HBPs were shown to improve color development and compatibility with polar oils. Benefits, such as long wear, improved formula aesthetics, lower pour temperatures, and greater structuring ability, is achieved by incorporating the HBPs into color cosmetic formulations.

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NOVEL DATA ON THE IMPACT OF WHITE BASE INGREDIENTS CHOICE IN MEETING THE PERFORMANCE BRIEF FOR LIPSTICK FORMULATIONS

Rebecca Peevers, Graham Pennick, Mike Hindley, Debra Jones and Adam Triantis

Croda Europe Ltd, East Yorkshire, England

Objective

There is little information known about how different waxes commonly found in lipsticks solidify different oils. The objective of this investigation is to study the relationship between commonly used waxes (Candelilla; Carnuba and Ceresin Wax) and a selection of oils, focussing specifically on the firmness of the base and correlating this to the hardness and pay-off of the fully formulated stick.

Methodology

For this investigation a number of oils were assessed, all of which are used in lipstick formula. All assessments were compared with Castor Oil and to appropriate off the shelf product equivalents.

Table I List of the oils used in the investigation

Ref.	INCI name	Ref.	INCI name
A	Castor Oil	K	Pentaerythrityl Tetracaprylate/Caprate
B	PPG-15 Stearyl Ether	L	PPG-3 Benzyl Ether Myristate
C	Propylene Glycol Isostearate	M	Isotridecyl Isononanoate
D	Isostearyl Isostearate	N	Di-PPG-2 Myreth-10 Adipate
E	Isostearyl Alcohol	O	PCA Dimethicone
F	Triisostearin	P	Pentaerythrityl Isostearate/Caprate/Caprylate/Adipate
G	Trimethylolpropane Triisostearate	Q	PPG-3 Hydrogenated Castor Oil
H	Polyglyceryl-3 Diisostearate	R	Di-PPG-3 Myristyl Ether Adipate
I	Ethylhexyl Cocoate	S	Pentaerythrityl Tetraisostearate
J	Ethylhexyl Pelargonate		

Initial screening looked at oil/wax interaction using a TA.XTPlus Texture Analyser and Spreadability Rig Accessory. The 3 commonly used waxes were assessed separately with the oils listed in Table 1 and a combination of all 3 waxes. The assessment measured the increasing force of the oil/wax blend up to a maximum penetration depth of the cone.

To assess the hardness of a fully formulated stick the texture analyser was used again this time using a cantilever attachment, measuring the point at which the stick breaks away from the main body.

For the pay-off assessment a mechanical arm apparatus was utilised. Under a set force a lipstick segment was moved back and fourth across a substrate, the weight of the deposition was taken as a measurement of pay-off.

Results

From the 19 oils assessed for oil/wax interaction, a range of firmness from 2500g to over 9500g was shown, Castor Oil proved to be a high firming oil (9000g) along with Di-PPG-2 Myreth-10 Adipate (8600g), Polyglyceryl-3 Diisosoterate (7600g) and PPG-3 Hydrogenated Castor Oil (6400g). Isostearyl Alcohol was proven to be a low structuring oil at 2500g.

To correlate this with a fully formulated stick, further assessments looking at the breakage point of the stick were completed proving that a higher oil/wax interaction provides a higher breakage point to the stick. **Figure 1** shows clearly that Castor oil (A) and Di-PPG-2 Myreth-10 Adipate (N) produce a very strong stick with breakage point values of over 700g. PPG-3 Hydrogenated Castor Oil (Q) and Polyglyceryl-3 Diisostearate (H) also offer a high stick strength with values over 500g. As expected from the initial oil/wax interaction results Isostearyl Alcohol (E) offers a low breakage point value of 350g. The red bar indicated the breakage point range of the assessed off the shelf products.

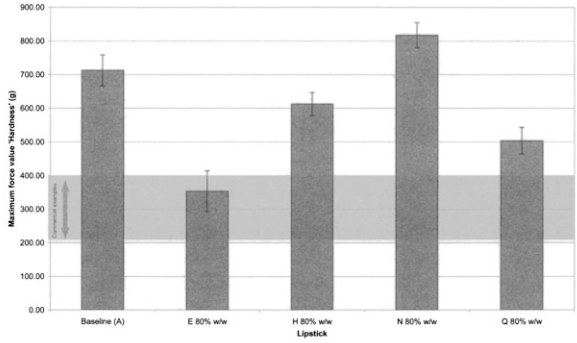

Figure 1 Lipstick 'Hardness' data using a TA.XT Plus Texture Analyser and Cantilever Rig. Chart of lipstick hardness for formulations containing complete replacements of Castor Oil

Finally the sticks were assessed for pay-off, which was generally found to be inversely proportional to the sticks firmness, however some interesting exceptions were found. **Figure 2** shows that Castor oil (A) and Di-PPG-2 Myreth-10 Adipate (N) both of which produce a very strong stick also offer very low pay-off as predicted. However Polyglyceryl-3 Diisostearate (H) and PPG-3 Hydrogenated Castor Oil (Q) did not follow this trend and instead gave a relatively firm lipstick which also gave a high level of pay-off.

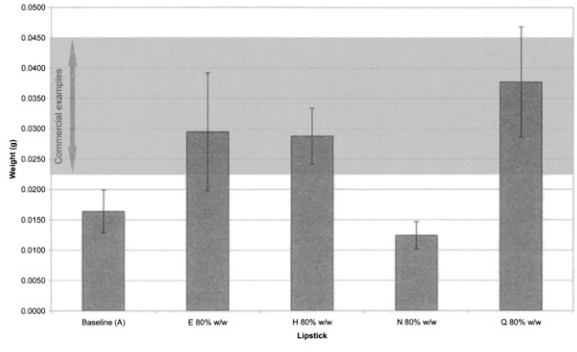

Figure 2 Lipstick 'Pay-off' data using mechanical arm apparatus. Chart of lipstick pay-off for formulations containing complete replacements of Castor Oil.

Conclusion

The use of oil/wax interaction data through instrumental analysis can be used to predict lipstick formulation firmness. By selecting high structure forming oils from the oil/wax interaction data, and testing these with the cantilever rig in a fully formulated lipstick, it can be seen that several of the oils formed very good oil/wax structures and hence can be used to increase the hardness of soft lipstick formulations. A clear trend was seen that links the firmness of the lipsticks inversely to the level of pay-off allowing both firmness and pay-off to be controlled by altering the formulation as required.

HAND SANITIZER FORMULATIONS WITH IMPROVED MOISTURIZATION

Lisa A. Flugge-Berendes, Ph.D., Jeffrey R. Seidling, Scott W. Wenzel, Corey T. Cunningham, Ph.D., Barbara J. Dvoracek, Melissa Martinez, Cindy J. Gould and Andrew D. Basehoar

Kimberly-Clark Corporation, 2100 Winchester Road, Neenah, WI 54956

OBJECTIVE

Alcohol hand sanitizers can be drying and irritating to the skin with repeated usage. This represents a significant barrier to compliant usage in health care settings where skin irritation can be so severe that healthcare workers ignore CDC recommended hygiene practices to prevent further skin damage.[1] The objective of this study was to develop a line of hand sanitizer formulations, including an alcohol gel and non-aerosol pump-foaming alcohol solution, which contain humectant and occlusive ingredients to counteract the drying effect of alcohol and offer superior moisturization to competitive market offerings.

Table 1: Composition (INCI Names) of Non-aerosol Foaming and Gel Alcohol Hand Sanitizer Formulations.

Non-aerosol Foaming Alcohol Sanitizer	Alcohol Gel Sanitizer
Alcohol	Alcohol
Water/Aqua	Water/Aqua
PEG-10 Dimethicone	Glycerin
Meadowfoamamidopropyl Betaine	Carbomer
Betaine	Petrolatum (and) Dimethicone (and) Ceteth-10 (and) Steareth-21 (and) Poloxamer 335
PEG-7 Glyceryl Cocoate	Aminomethyl Propanol
Fragrance	Fragrance
–	Panthenol
–	Tocopheryl Acetate
–	Hydroxypropylcellulose

METHODOLOGY

Moisturizing ingredients compatible with a gel sanitizer and a foam sanitizer were identified. The compositions of the gel and foam hand sanitizer are detailed in Table 1. The gel contains emollient and occlusive ingredients that ordinarily are not compatible with hydroalcoholic systems; the new formulation is patent pending. The foaming composition contains ingredients that form dense foam upon application and also provides significant moisturization; the new formulation is patent pending. Both a foam and gel hand sanitizer were tested for moisturization against competitive products in separate 20 person clinical studies. Moisturization levels were determined using the industry standard of conductance. Conductance measurements were taken before application and at 30 and 120 minutes post application on the forearm to determine moisturization levels.

RESULTS

Conductance measurements on both the gel and the foam formulations showed statistically significant improvements over baseline moisturization after two hours, and statistically improved moisturization (at 95% confidence) over most of the competitive codes. Only one competitive code had moisturization at statistical parity to the new gel formulation (Figure 1); the new foam formulation provided improved moisturization over all other competitive codes (Figure 2).

Table 2: Identification of codes

Code	Description		Code	Description
C	Untreated site		U	Untreated
G	**Alcohol Gel Sanitizer**		K	**Non-aerosol Foaming Alcohol Sanitizer**
E	Competitive Moisturizing Alcohol Gel 1		Q	Competitive Moisturizing Alcohol Foam 1
D	Competitive Moisturizing Alcohol Gel 2		H	Competitive Moisturizing Alcohol Foam 2
R	Competitive Moisturizing Alcohol Gel 3		A	Competitive Moisturizing Alcohol Foam 3
S	Competitive Moisturizing Alcohol Gel 4			
P	Competitive Alcohol Gel without Moisturizers			

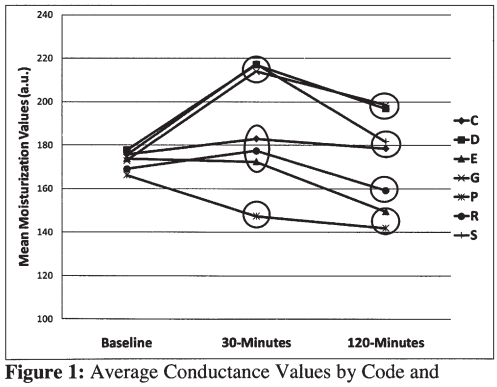

Figure 1: Average Conductance Values by Code and Time. Circles indicate statistical significance groupings at 30 and 120 minutes based on least squares mean change from baseline at 95% confidence interval.

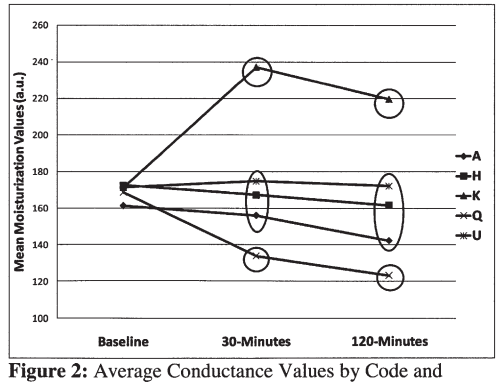

Figure 2: Average Conductance Values by Code and Time. Circles indicate statistical significance groupings at 30 and 120 minutes based on least squares mean change from baseline at 95% confidence interval.

CONCLUSION

The data in this study prove that moisturizing ingredients can be incorporated and effectively delivered in ethanol based foaming and gel sanitizers. Gel and moisturizing foam prototypes provide significant moisturization benefits that last for at least 2 hours. Only one competitive gel product had statistically equivalent benefits to the prototype moisturizing gel and no foaming sanitizer was in the same statistical grouping as the prototype foam sanitizer. Several competitive products did not provide a moisturization benefit at all and some, in fact, were drying.

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LOW-ENERGY EMULSIFICATION, VII: IMPROVING EMULSION QUALITY BY CONTROLLING PHASE-INVERSION AND REDUCING ENERGY APPLICATION

T. Joseph Lin[1], Ph.D., Jennifer Chang[2] and Gregory Pum[2]

[1]*628 Enchanted Way, Pacific Palisades, CA 90292*
[2]*Levlad, Inc., Chatsworth, CA 91311*

Introduction
Various types of emulsions have important applications in a wide range of cosmetic preparations, including skin care, hair care, sun care and makeup products. In most cosmetic applications such as skin creams and moisturizing lotions, product viscosity is relatively high and a good number of synthetic polymers or natural gums can be incorporated to help stabilize the product against phase separation and other signs of quality degradation. However, in some applications, such as sprayable sunscreens or tanning emulsions, the viscosity must be very low to allow uniform spraying, and traditional polymers or thickeners cannot be used to assure product stability. Such products will usually require formulating emulsions with very small droplet sizes. Although microemulsions have extremely small particle sizes (generally less than 0.14 micron), they are usually not suitable for this purpose, because they require the use of a large amount of surfactants, which could cause sticky feel on application or promote skin irritation. Generally, very fine o/w emulsions having average droplet diameters no larger than a few microns and viscosities less than 50 cps are used in such products.

Emulsification by PIT Method
A popular way to formulate and manufacture this kind of low-viscosity emulsion is via a phase inversion temperature (PIT) method where carefully selected nonionic surfactants serve as emulsifiers to make fine o/w emulsions at a temperature near PIT. It is known that interfacial tension between the oil and water can drop significantly when a selected mixture, consisting of water, oil and certain nonionic surfactants are heated to a temperature near PIT (1). Emulsions having very small droplets can be formed when this mixture is stirred, even without using a large amount of surfactants or a high-shear mixer. However, in using this method, the emulsion must be cooled fairly quickly from the PIT zone to a lower temperature to avoid coalescence of emulsion droplets, resulting in formation of a coarse emulsion and unstable product.

Processing Problem for Large Batches
Making this kind of an emulsion in the laboratory is relatively easy, because the cooling of the emulsion in a small beaker is quick even without using a water bath. Making a small, 50-gallon pilot batch of such formulation is also not difficult, because cold water can be circulated in the kettle jacket to promote cooling and avoid droplet coalescence near PIT. However, to reduce production costs in a very competitive market, many cosmetic manufacturers have increased the size of their production batches, with, using 2000-gallon or even 5000-gallon kettles, significantly increasing the time needed to heat and cool the batches. For many cosmetic emulsions, increased cooling time will not negatively affect the product quality. However, for some emulsions, including fine-droplet size emulsions processed with a PIT method, slowed cooling rate can cause droplet coalescence and significant quality degradation.

The speed of heating or cooling in a batch kettle is affected by heat-transfer efficiency and the rate of heat transfer per unit area is known as the "heat flux" which can be expressed in BTU per square foot per minute. The local heat flux, dq/dA can be related to temperature driving force, $(T_h - T_c)$ where T_h is the average temperature of the hot emulsion and T_c is that of the cooling water in the jacket by following equation:

$$dq/dA = U\,(T_h - T_c) = U\ \Delta T$$

The proportionality factor, U, is also known as the local overall heat transfer coefficient and ΔT represents temperature driving force, $(T_h - T_c)$. The above differential equation can be integrated over the entire surface area to calculate the total amount of heat transfer. When batch size is increased by processing the emulsion in a larger kettle, the surface area available for heat transfer per unit volume of the batch will decrease proportionally, if both kettles are similar in shape. In addition, the thicker metal needed in constructing the larger kettle will increase resistance to heat transfer and can reduce cooling efficiency. The result of slower cooling can cause product degradation in emulsions made with a PIT method. One way to increase cooling rate is to reduce the temperature of cooling water in the jacket, but this can be expensive

and not always very effective. Another way is to greatly increase the surface area of heat transfer by using a heat exchanger. However, purchasing a new heat exchanger can also be an expensive investment.

Application of Low-Energy Emulsification (LEE) Method

Low-Energy Emulsification (LEE) was originally introduced by Lin to reduce both energy consumption and processing time in manufacturing cosmetic emulsions (2). The principle of LEE is quite simple. It is based on realization that conventional manufacturing of cosmetic emulsions uses far more energy than is necessary, and that much energy and processing time is wasted in cooling the product. Conventional manufacturing of cosmetic emulsions generally involve heating both the aqueous and oil phases to about 80°C before combining them. The freshly formed emulsions are generally cooled to near room temperature before being stored for filling. As the batch size is increased, the time required for batch cooling also increases significantly. To speed this process, many manufacturers use refrigerated fluid for cooling, basically spending more energy to discard energy.

Most cosmetic emulsions contain a large portion of water (60 – 90%) in the formulation. Much energy can be saved by heating only a part of the water phase to make an emulsion concentrate and adding the remaining water in the formula cold to cool the batch. Of course, there are significant details to consider in implementing this concept, but also many possible variations of LEE procedures which can be adopted to process a wide range of cosmetic emulsions. It has been demonstrated that careful application of energy only where and when needed can significantly lower energy use and reduce processing time without affecting product quality (3, 4, 5, 6, 7). The purpose of this study was to find the best way to apply LEE procedure to accelerate cooling rate during the critical stage of emulsification to prevent product degradation in processing large batches of low viscosity, PIT emulsions.

Experimental Results

A temperature-controlled emulsification apparatus was constructed to accurately simulate slow cooling rates encountered in large batch production. By carefully varying the rates of cooling in the critical temperature zone near PIT and observing the effects on emulsion properties, data were obtained to allow precise determination of cooling rate required to maintain emulsion quality. The experiments were repeated using different formulations to compare the effects of using conventional emulsification procedure and modified LEE procedures. Control of cooling rate was found crucial in preventing degradation. It was discovered that when the cooling rate dropped below a certain critical value, the emulsion quality definitely suffered, as indicated by the presence of large droplets observed under microscope. The main reasons for degradation are believed to be incomplete phase inversion at PIT zone and droplet coalescence caused by slow cooling. The modified LEE procedure can effectively prevent quality degradation in large size batch production.

Conclusion

The experimental data clearly demonstrated another useful application of LEE technique in processing emulsions. When correctly executed, it can substantially reduce both processing time and energy cost in large batch production, while improving emulsion quality and significantly reducing carbon footprint.

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(7) T. J. Lin, *Manufacturing Cosmetic Emulsions: Pragmatic Troubleshooting and Energy Conservation*, Allured Books, (*2009*)

ENHANCING DYE PENETRATION AND COLOR DEVELOPMENT IN LIQUID OXIDATIVE HAIR DYE FORMULATIONS

Timothy Gao, Ph.D., Charles Moses and Jung-Mei Tien

Croda, Inc., 300-A Columbus Circle, Edison, NJ 08837

INTRODUCTION

Phosphate esters have long been used in hair and skin care formulations including hair relaxers, hair perms, sunscreens, and color bases as excellent emulsifiers and viscosity thickening agents. In previous study (1), we reported that MLV structure formed in sunscreen formulas with a phosphate ester emulsifier played an important role enhancing the deposition of sunscreen oil on the skin surface, and therefore, improving the SPF water-wash resistance. It was also found that addition of phosphate esters into hair dye base formulas enhanced formation of multilayer lamellar vesicles (MLV) and improved their coloring performance. The molecular structure of added phosphate ester showed significant effects on the formation of MLV phases and rheological properties (2-3).

In this paper, we report our recent studies on developing better and faster liquid/cream oxidative dye formulas with and without additional Hair Color Enhancer (HCE – Oleth-5 Phosphate and Dioleyl Phosphate). Hair color intensity, dynamic advance contact angle of dyed hair, and color wash fastness of dyed hair samples were determined and compared. Image analysis on cross-sectional areas of dyed hair samples was also performed

EXPERIMENTAL

- ***Materials:*** Liquid oxidative dye formulas (Auburn and Black) with and without additional Hair Color Enhancer were used to dye regular bleached hair for 10 and 20 minutes.

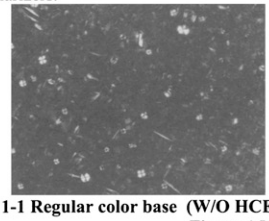
Regular bleached hair was purchased from International Hair Importers, Inc. (NY)

- ***Instruments:*** A LabScan XE spectrocolorimeter for measurements of color indexes; A Kruss K100 automatic Tensiometer for measurements of dynamic advancing contact angle of single hair fibers; A Leica RM 2155 Microtome for cross-sectioning hair fibers; and a Nikon Optiphot-Pol optical microscope with digital camera and Image Analysis for imaging of liquid crystals in hair dye formulas and cross-sectional areas of dyed hair fibers.

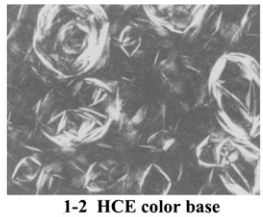
RESULTS AND DISCUSSION

1. Emulsion structure of two color formulas

Figure 1 shows digital images of two hair dye (Auburn) samples under the microscope with crossed polarizers.



1-1 Regular color base (W/O HCE)



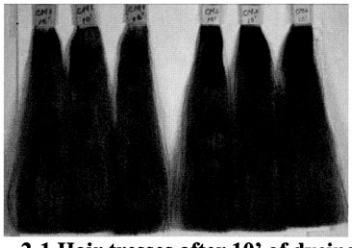
1-2 HCE color base

Figure 1 Micrograph of different color bases (X100)

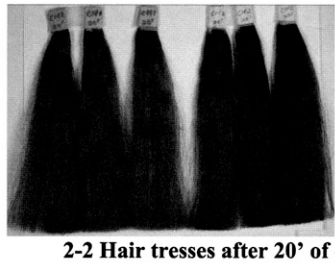
It is observed that HCE color base formed very large crystal structure, while the color base W/O HCE had very tiny crystals. This observation is consistent with our previous study (2).

2. Differences in color developing rate and final color strength

Figures 2-1 and 2-2 explain color differences in hair tresses dyed with these two Auburn formulas at 10 and 20 minutes, respectively. It is apparent that hair tresses dyed with HCE formula (CM2) demonstrated darker and stronger color intensity than those dyed with the formula W/O HCE (CM1) after 10 and 20 minutes. The calculated changes in color indexes further validated visual observations. Table 1 lists changes in color indexes of dyed hair tresses after 10 and 20 minutes of dyeing using different hair dye formulas. Values of total color change (ΔE) in hair tresses dyed by HCE-containing formula are always larger than those of hair dyed with formula W/O HCE at the same dyeing time. It is evident that additional HCE not only accelerated hair dyeing rate, but also improved the final color strength.



2-1 Hair tresses after 10' of dyeing



2-2 Hair tresses after 20' of dyeing

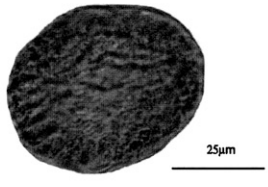
Figure 2 Dyed hair tresses at different dyeing time with different hair dye formulas (CM1 – Hair dye W/O HCE; CM2 – Hair dye with HCE)

Table 1 Change in Color Indexes of Dyed Hair Tresses

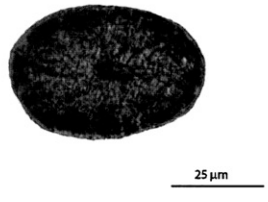
Hair Dyeing	ΔL	Δa*	Δb	ΔC	ΔE
CM1-10'	-36.48	13.36	-5.89	14.60	39.73
CM1-20'	-39.40	13.67	-7.72	15.70	43.11
CM2-10'	-39.67	12.49	-8.12	14.90	43.14
CM2-20'	-43.20	9.29	-11.62	14.88	47.14

3. Images of Cross-sectional areas of dyed hair fibers

Typical images of cross-sectional areas of hair fibers dyed with two different dye formulas (black) are shown in Figure 3.



3-1 Hair after 20' of dyeing W/O HCE



3-2 Hair after 20' of dyeing with HCE

Figure 3 Cross-sectional areas of dyed hair fibers with different dye formulas

It can be seen that the cross-sectional area of hair fiber dyed with the formula containing HCE showed denser dark dye granules than the hair dyed with the corresponding formula W/O HCE. It is clear that addition of HCE in the formula improved dye penetration inside hair.

CONCLUSIONS

- Addition of HCE into oxidative hair dye formulas changed their emulsion structure, accelerated dye penetration rate inside hair, and made the hair dyeing process faster and hair color strength stronger.

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COSMETIC COLOR ADDITIVES: AN OVERVIEW AND COMMON MISUNDERSTANDINGS

Donald C. Havery and Shontell Fleming Wright

U.S. Food and Drug Administration, College Park, Maryland

Introduction

Under U.S. law, cosmetics and their ingredients are not subject to premarket approval by the Food and Drug Administration (FDA), with the exception of color additives. FDA prohibits the sale or import of cosmetics containing color additives that have not been approved, have not been certified (if required), or are present in product types for which they have not been approved. This presentation will review FDA's color additive regulations and the most common errors made by cosmetic manufacturers with respect to color additives.

Historical

Artificial color additives were first used in the late 1800s when they were added to foods such as butter and cheese. These colors were synthetic organic compounds that were derived from coal tar. Some of these colors were probably toxic and may have been added to hide inferior food. This situation prompted the involvement of the Federal government and led to the establishment of the color additive regulations.

In 1906 the Food and Drugs Act was enacted which prohibited poisonous colors in confectionary. By 1907 seven color additives were approved for use in food; by 1931 this number had increased to fifteen. Significant changes occurred in the regulation of cosmetics and color additives with the passage of the 1938 Federal Food, Drug, and Cosmetic Act (Act). The Act increased government regulatory authority by adding FDA responsibility for oversight of cosmetics. The Act also mandated the approval (listing) of coal tar colors suitable for food, drugs and cosmetics, and included mandatory certification of batches of some color additives. Under the authority of the Act, FDA created procedures for color additive approval.

Additional changes to the regulation of color additives occurred in 1960 with the passage of the Color Additive Amendments. These changes included: creation of a formal definition for "color additive": updates to the color additive petition process: and introduction of the Delaney Clause, which prohibits the use of carcinogens (to man or animal) in food, drugs or cosmetics.

Color Additive Regulations

As noted above, all color additives are subject to premarket approval. Approved color additives are listed in Title 21 of the Code of Federal Regulations, Parts 73, 74, and 82. Each color additive listing includes the name of the color additive, specifications, uses and restrictions, labeling, and certification information. Specifications include information on color purity, intermediates (starting materials), other colored impurities, salts, volatile matter, extractable matter, and heavy metals. Some color additives are only approved for specific uses and some have established maximum use levels. For example, some color additives are approved for general use (no restrictions), while others cannot be used in products that contact mucous membranes such as lipstick. Cosmetic products used in the area of the eye are unique in that only color additives that are specifically approved for eye area use in the listing regulations can be used.

There are no "Generally Recognized As Safe" color additives. The only exceptions to FDA's color additive requirements are coal tar colors used in hair dyes, which do not require premarket approval and are not considered to adulterate a hair dye as long as a specified caution statement and directions for a preliminary patch test appear in the product labeling.

What is a Color Additive?

A color additive is defined in the Act and regulations as:. ….. a dye, pigment, or other substance, made by a process of synthesis …, or extracted, isolated, or otherwise derived, … from a vegetable, animal, mineral, or other source, and ….. when added or applied to a food, drug, cosmetic, or to the human body, ….. is capable (alone or through reaction with another substance) of imparting a color thereto. (section 201(t) of the Act, 21 CFR 70.3(f))

Included in the definition are white, black and gray. A material that otherwise meets the definition of a color additive may be exempt from the color additive listing requirements if it is being used for a purpose other than coloring. In that case, the color imparted by the material must be clearly unimportant to the appearance, value, marketability, or consumer acceptability of the product.

The regulations distinguish between two types of color additives, certified and certification exempt. Certified colors are synthetic organic dyes, lakes, or pigments obtained from petroleum or coal sources (eg. FD&C Blue No. 1). These color additives are submitted to FDA for batch certification to assure that they comply with the listing regulations for purity and composition. Color additives that are exempt from certification are primarily derived from plant or mineral sources (eg. iron oxides).

Common Errors

The following are examples of possible color additive errors:

1. **Using unapproved color additives**:

Fruit extracts (Only approved for food use)
Glitter
Sudan I, II, III, and IV (known carcinogens)
Phenolsulfonphthalein
Minerals (Gold, tourmaline, diamond, malachite, sapphire powder)
Astaxanthin
Erythrulose
Alumina
Calcium aluminum borosilicate
Mica-based pearlescent pigments (Only approved for food, drug, and contact lens use)
Botanical or nut extracts (licopene, walnut extract, chamomile extract, chlorophyll)
Activated charcoal
Tin oxide
Melanin
Azulene
Calcium sodium borosilicate
p-Phenylenediamine ("black henna")

2. **Using ingredients in product types for which they are not approved**:

. *Eye area*: Only color additives specifically approved for use in the eye area can be used in eye area products.
. *Externally Applied*: Color additives approved for externally applied cosmetics only cannot be used in products that are applied to mucous membranes such as the lips. For example,
  . Ferric ferrocyanide is not approved for use in lipstick
  . Chlorophyllin-copper complex is only approved for dentrifices
  . Aluminum powder is not approved for lipstick

3. **Using color additives that are only approved in the EU**:

. Barium sulfate
. Stannous chloride

Conclusion

It is important for cosmetic manufacturers to be aware of the color additive regulations. Be sure that the color additive is approved for the product's intended use. Be aware of any concentration limitations. When purchasing color additives subject to certification, check the label to be sure it has a certification lot number.

References

Summary of color additives approved for use in the US:
http://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditiveInventories/ucm115641.htm#table3A
Color Additives and Cosmetics:
http://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditivesinSpecificProducts/InCosmetics/ucm110032.htm
History of color additives
http://www.fda.gov/ForIndustry/ColorAdditives/RegulatoryProcessHistoricalPerspectives/default.htm

VISUALIZATION OF HAIR COMBING FRICTION UTILIZING THERMAL IMAGING

Renee Bolden[1], Ph.D., Dirk Domaschko[1], Jeni Thomas[1], Ph.D., Mark Brown[1], Marge Peffly[1], Ph.D., Jennifer Marsh[1], Ph.D. and Yujun Li[2], Ph.D.

[1]*The Procter & Gamble Company, Sharon Woods Innovation Center, Cincinnati, Ohio*
[2]*The Procter & Gamble Company, Beijing Technical Center, China*

Background

Historically, thermal imaging has been used to visualize heating and cooling events in various settings such as plant processing, product application on skin or even hot body detection by the military. We know that when an increase in friction occurs there is a heat event that corresponds (such as rubbing your hands together to keep them warm when cold). We have drawn a parallel between this example and hair damage to visualize product performance. If your hair is damaged or unconditioned the force it takes to comb your hair increases causing friction between the comb and hair. In Hair Care, dry hair conditioning benefits are typically quantified using technical friction methods such as sled or combing friction. Using thermal imaging we have optimized a method to visualize and quantify this increase in combing friction based on the thermal change. This method is unique because it provides an engaging visual to use for communicating product differences as well as quantitative data that can be used to statistically differentiate product performance benefits. In addition, the method is extremely versatile since the camera is portable and can be used in a wide range of settings.

Method

To conduct the experiments, hair switches (4gm, 8 inch) were hung from a stand and combed once for detangling. For the test, each switch was combed at a constant rate. A metronome was used to keep the combing rate equal between hair switches and a single person was used to comb all switches within a study to account for person to person combing variations. Each formulation was tested for n=3 replicates. The combing event was captured in real time using a Mikron Thermal Imaging camera. For each sample, three pieces of data were obtained: 1) visual image of temperature increase, 2) quantitative temperature data and 3) rate of temperature increase. Using the Mikrospec software two single images were extracted from the experiment movie. The average change in temperature was then determined for a specific region of interest (ROI) on the hair switch at the initial (before combing) and end (after continuous combing) of the sample. The ROI selected was a rectangle (size kept constant between samples) down the center of the hair switch where the most change in combing friction was observed. From this data, the total change in temperature (final – initial) was calculated for each sample and then later compared to a non-conditioning shampoo. The temperature comparison calculation to determine the benefit attained versus a non-conditioning shampoo was: (100% - (shampoo/non-conditioning control * 100)). The rate of temperature increase was determined from the slope of a ROI temperature versus time plot. In addition to quantitative data, avi movies of the each combing experiment were created to create side by side visual comparisons.

Results

Thermal imaging was used to evaluate several shampoo and conditioner formulations across various hair types (virgin and bleached). For virgin hair, differences in combing friction were measured comparing switches treated with a non-conditioning shampoo, shampoo prototype designed to smooth frizzy hair and currently marketed smoothing-benefit shampoo. We found that the benefits of the prototype formula could be differentiated from the non-conditioning shampoo and the currently marketed product with this method. The prototype decreased the combing friction 62% versus the non-conditioning shampoo and 72% versus the currently marketed shampoo (see Figure 1&2). In addition to the decrease is overall change in temperature, the smooth shampoo prototype had a slower rate of temperature increase as seen in Figure 3.

Figure 1 – Virgin Hair Shampoo Comparison

Figure 2 – Virgin Hair Shampoo

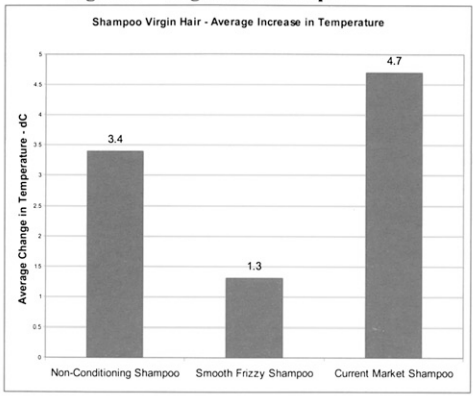
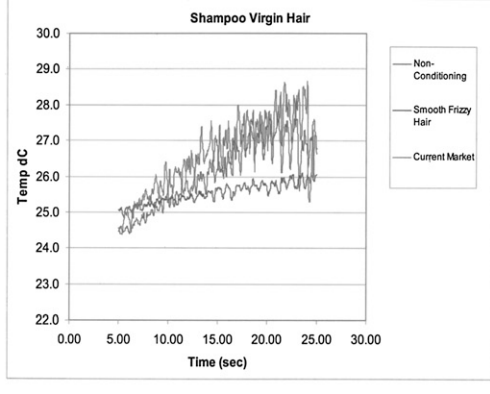


Figure 3 – Virgin Hair Temperature Rate



For bleached hair, shampoos typically do not deposit silicone on the surface due to the change in hair surface polarity from color damage (hydrophobic to hydrophilic) and therefore combing friction will not typically be significantly reduced. Using thermography, we compared a non-conditioning shampoo, currently marketed smooth shampoo and shampoo prototype to smooth color-treated hair containing poly-DADMAC. We found that the friction-reducing benefit of prototype could be differentiated from both the non-conditioning shampoo and the currently marketed product. The prototype reduced the combing friction 53% versus the non-conditioning shampoo supporting the data that poly-DADMAC-containing formula provides reduced combing friction via an increase amount of silicone being deposited. The current shampoo only reduced the friction by 35% versus the non-conditioning shampoo.

Figure 4 – Bleached Hair Shampoo Comparison

A STUDY ON THE INFLUENCE OF SURFACTANTS ON HAIR COLOR FADING

Jon Kiplinger, D. Bendejacq, E. Leroy and M. Adamy

Rhodia, Inc., 8 Cedar Brook Drive, Cranbury, NJ 08512-7500

Background:

Protection of hair color is a need a growing importance among consumers. The growing use of hair colorants is mostly driven by the ageing of the population and the wish to give an attractive look to hair. Color protection shampoos have been around for more than 20 years and there are a number of publications on that matter. However, while a lot of attention has been paid on the benefits of conditioning polymers and silicone oils, very little was ever published on surfactants. In this study we have performed different series of color fading experiments, investigating the protective (or destructive) potential of surfactants, as well as commercial benchmarks. We have paid special attention to a strong trend in North America where a growing number of shampoos are formulated sulphate-free or SLS/SLES free.

Experimental:

Single bleached or double bleached hair tresses were collected and dyed using a L'Oreal Preference Auburn. Their initial colour after dyeing was determined using a spectrocolorimeter, giving tresses Lab coordinates. The loss of color upon washing cycles (1, 3, 5 or more) has been defined according to the following formula: $\Delta E=\sqrt{(\Delta L^2+\Delta a^2+\Delta b^2)}$.

Using all L, a and b values of a set of tresses, we compute the average $L_{av}a_{av}b_{av}$ color of the entire collection of hair tresses and compute the color difference $\Delta E_i=\sqrt{((L_i-L_{av})^2+(a_i-a_{av})^2+(b_i-b_{av})^2)}$ that each tress "*i*" has with respect to this central color. This leaves us with a collection of ΔE_i measurements, whose distribution (see Figure 1) gives an overview of how close the tresses are among this collection. All hair tresses whose "distance" to the central color was too large was considered an ill point as schematically represented in Figure 2. We only selected tresses closest in terms of initial color, with an average difference not larger than a given threshold.

Depending on how homogeneous the colouring process was, and on how strict the thresholding was, up to 50% of the hair tresses can be discarded.

Figure 1

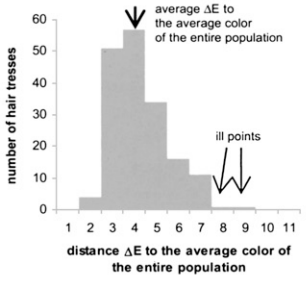
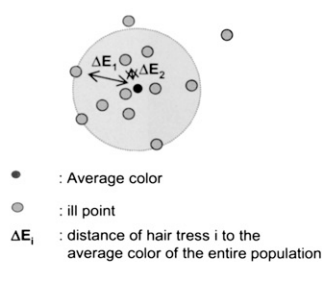


Figure 2



Results and Discussion:

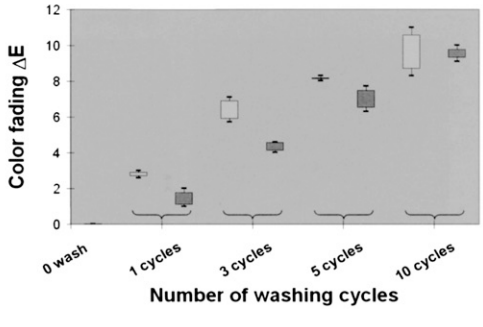
Any hair dye, even permanent, is vulnerable to washout. The primary cause of color fade is the dye washout caused by water itself. Even one washing cycle on double bleached Caucasian hair can result in a ΔE of 10 units. In addition, the more damaged the hair (function of the number of bleaching cycles) prior to the coloring process, the higher the ΔE value regardless of the number of washing cycles.

Several surfactants were tested and benchmarked against sodium lauryl sulfate (SLS) and sodium lauryl ether sulfate 2EO (SLES-2). A commercial daily use shampoo was also used as a first point of reference in the study.

The extent of color fading caused by the commercial shampoo was found to be very similar to the SLES-2 at 16% active up to 10 washes.

Figure 3 shows the results for Na N-methyl cocoyl taurate vs. SLES-2 as the benchmark. The taurate showed superior performance at 1 – 5 washes, but by the time 10 washing cycles were performed, there was no statistical difference. When testing the surfactants, it was found that better differentiation was obtained by limiting the number of wash cycles to 3. Since these were simple letdowns of the test surfactant in water adjusted to pH 6.0 with citric acid, we hypothesized that after many cycles the effect of water itself on color fading would eventually overwhelm that of any other surfactant ingredients of the solution.

Figure 3 – Extent of color fading as a function of the number of washing cycles, caused by 16wt%-solutions based on Na Methyl Cocoyl Taurate ■ or SLES-2 □.



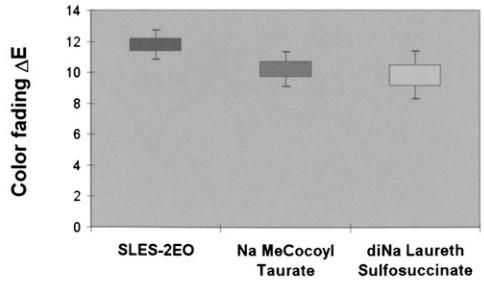
Secondary surfactants such as taurates and sulfosuccinates showed a reduced color fading compared to SLES-2. The results are shown in Figure 4.

Combinations of 12% SLES-2 with 4% Na LA or Na CA also showed a reduction in color fade vs. the sulfate alone after 3 washes. This study was done on single bleached tresses, and showed a 26% reduction in ΔE. This data appears in Table 1 hereafter.

Table 1: SLES-2 + Amphoteric

Surfactant (s)	ΔE (mean)
SLES-2 (16%)	5.78
SLES-2 + Na Lauroamphoacetate (12:4)	4.3

Figure 4 – Secondary surfactants. Extent of color fading after 3 washing cycles, caused by SLES-2EOs-, taurate- or sulfosuccinate-based 16wt% solutions.



Designing shampoo bases to minimize color fading can be challenging. Due to the poor performance of sulfates in general for color fade, it is better to formulate sulfate free for hair color preservation. A sulfate free (SF) system and a sulfate free commercial shampoo were evaluated against SLS, and were found to cause less color loss in dyed hair. This is an important emerging industry trend, and one of the main drivers for formulating sulfate free shampoos. (see Figure 5).

However, a structured liquid system (multi- lamellar vesicles) based on Na Trideceth Sulfate provided ~50% less color loss following 3 washes than the identical system not structured (i.e. in a micellar phase) (see Figure 6).

Figure 5 – Sulfate-free system vs. SLS

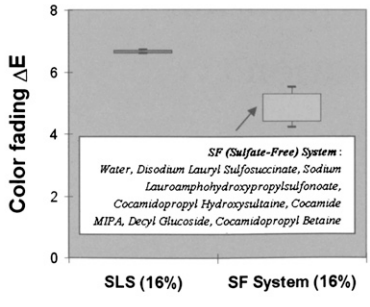
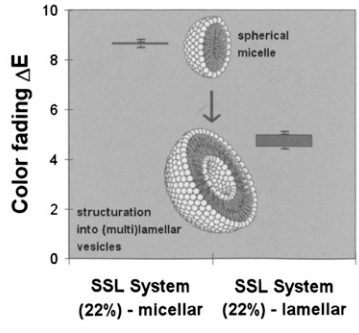


Figure 6 – Benefits of Structured Liquid Technology



Conclusion:

Several factors contribute to hair color fade from shampooing. While water itself has a big impact, it cannot be avoided in the shampooing process. This study indicates that avoiding sulfates in shampoo formulations can lead to improved color retention. The performance of sulfate surfactants can be improved by replacing sulfates with "milder" anionics such as taurates and sulfosuccinates and by adding an amphoteric co-surfactant.

Very interestingly, it has been found that the color fading effect of sulfates can be greatly reduced when formulating in a structured liquid phase.