J. Cosmet. Sci., 49, 185–212 (May/June 1998)

Preprints of the 1998 Annual Scientific Seminar

May 7–8, 1998
Westin Hotel
Seattle, WA

COMPARISON OF FACIAL CHARACTERISTICS OF JAPANESE AND CAUCASIAN SKIN

Amy C. Zimmerman, Albert M Kligman, M.D., Ph. D., Ronald L. Rizer, Ph. D, John V. Scimeca, James R. Mayne

Amway Corporation, Ada, MI 49355

INTRODUCTION

Traditional safety studies used to develop products have not been predictive of adverse results in the field, due to differences between Japanese and Caucasian skin. The difference between these two populations is centered around the fact that both Japanese dermatologists and the Japanese people perceive their skin to be sensitive. The Japanese population has exhibited more neuro-sensory reactions such as itching, burning and stinging as compared to Caucasian populations. Ordinary safety and use testing does not forecast these differences.
The development of products for a global marketplace requires understanding of these differences. If cosmetic scientists are to be successful in formulating efficacious products for the Japanese market, we must first examine the skin characteristics of their populations and their reaction to topical preparations. The challenge for our industry is to determine how to predict and forecast potential problems.

MARKETING EXPERIENCE

High on the list of consumer complaints in the Japanese market are burning, itching and stinging, especially on the face. Both raw materials used and formulation types have been identified as contributors to these irritation reactions.

Problematic Ingredients	Problematic Formulation Types
Alcohol	Highly Occlusive Creams
Preservative Systems	Some Leave-On Products
Fragrances	Masks
Low Molecular Weight Esters	Eye Area Products
Organic Sunscreens	

Another area to consider when marketing products to the Japanese is the fact that there are real cultural differences between the two populations. One area is in preference of product types. The Japanese prefer light products which go on smoothly and which leave little or no residue. Heavy, greasy or oily products are not well received. A second cultural difference is that the Japanese population is typically less concerned with facial wrinkles and lines. A much stronger focus is placed on the clarity, tone and texture of their skin. Minor changes in skin color or mottling of the skin are perceived as a very big problem.

Significant in determining the source of adverse reactions is the cultural differences in skin cleansing. The normal Japanese cleansing regimen includes the use of several products designed to remove makeup, thoroughly cleanse the skin and prepare the skin for moisturization. This cleansing routine is performed two or more times daily. This strong attention to completely cleansing the skin may result in subclinical irritation, thus making the skin increasingly sensitive towards otherwise innocuous ingredients and products. This is a prime example of Dr. Albert Kligman's concept of "Invisible Dermatology". The Japanese skin is already somewhat compromised by the cultural practice of extensive cleansing, so any new product introduced into the skin care regime may experience an unusual number of complaints in Japan.

CONCLUSIONS

Market experience has shown there are both real and perceived differences between Caucasian and Japanese populations. There is a real difference in the types of products preferred by the two populations, as well as the types of adverse reactions documented for these products. Sub clinical irritation due in part to extensive cleansing routines may play a large part in the increased rate of irritation.

Our clinical evaluations have shown many similarities between the two populations, but both directional and statistical differences were shown in several areas.
Japanese skin:

- **does appear to have differences in skin firmness and resiliency**
- **exhibits a decreased level of actinic damage as seen in higher Fitzpatrick scores, and probably a greater photoprotective capacity, due to increased levels of melanin**
- **has fewer fine lines and wrinkles due in part to safer practices regarding reduction of sun exposure**
- **exhibits a lower sebum secretion rate, inferring a reduction in acne prone skin**
- **possesses lower levels of ceramides 1 and 2**
- **differs in the skin surface lipid content from Caucasian skin**
- **shows apparent differences in microcirculation and blood flow response which may also be an indicator as to why there are variances in product safety studies of consumer test products when evaluated on Caucasian and Japanese panels for sale in the US and in Japan.**

ASIAN AND CAUCASIAN HAIR – DIFFERENCES OF INFLUENCE FOR THE FORMULATION?

J. Kahre[1], P. Bush[1], B. Salka[2], N. Totani[3] and W. Poly[3]

[1]Henkel KGaA, D-40191 Düsseldorf, Germany;[2] Henkel Corp., Ambler, PA 19002;
[3]Henkel Japan Ltd., Tokyo, Japan

Introduction:

Hair is undoubtedly one of the most important features of people in all cultures. For the past several centuries hair has played an important role. Style, length and color changes are influenced by fashion trends. The hair often allows for feelings of health and beauty, and thus its influence is of great importance. Therefore hair has been studied greatly as cited in numerous publications.Three major types of hair are known: african, asian and caucasian hair. The differences of the hair types are related to the diameter, geometry and other physical parameters. Closely related to these parameters are the biophysical factors, tensile strength and combing forces, which might be influenced by cosmetic formulations that are applied to hair. We examined asian and caucasian hair and applied shampoo and conditioner formulations in order to study their effects.

Experimental:

Biophysical measurements:
Asian and caucasian hair from Alkinco New York was used. Tensile strength and combing forces were measured using a robotic system as described in the literature (1/2).

Results:

A - Combing Work and Tensile Strength
The differences in the morphology (area, thickness, half axes) influence physical properties (see figure 1 and 2).

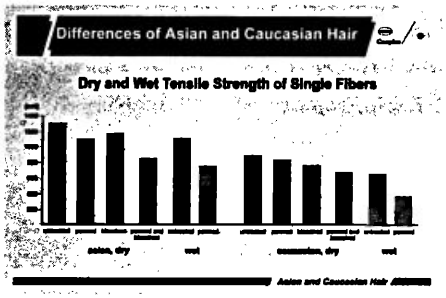
B - Application of different shampoos
The addition of protein hydrolysates increases the tensile strength of damaged hair of both types. A special shampoo for fine hair (94/145/11) shows differences in the objectively measured efficacy. It is best applicable on caucasian hair for which it was developed. It seems that a „world formulation" is possible by adjusting the concentration of quaternary polymers (see figure 3, 4 and 5).

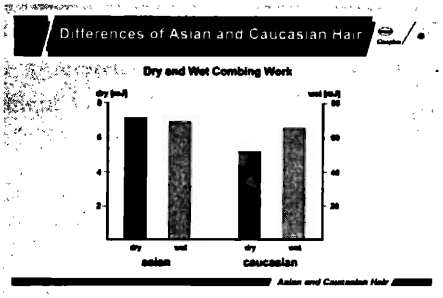
C - After Hair Treatments
The tested formulations have the same efficacy on both hair types. The addition of emollients shows more of a increase on asian hair than on caucasian hair (see figure 6 and 7).

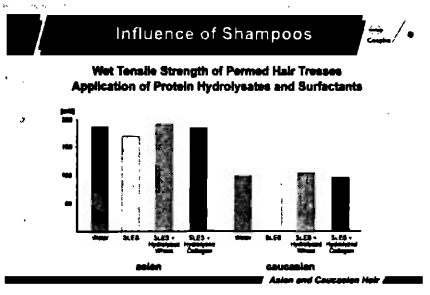
Conclusion:

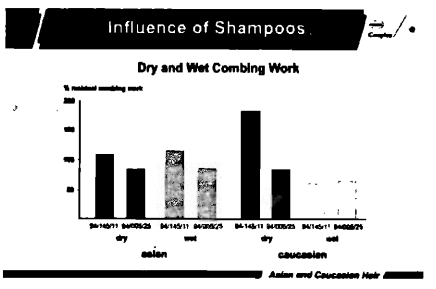
Due to the differences in the morphology we found differences in the biophysical behaviour of asian and caucasian hair. We also found differences in the efficacy of shampoo and conditioners. Therefore it is better to develop different products for different hair types.

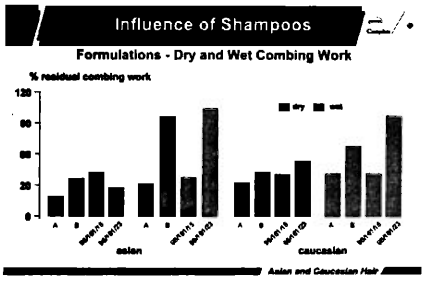
Literature
1) Busch, P., Thiele, K., Fischer, D.; *Seifen, Öle, Fette, Wachse* **108** 305-309 (1989)
2) Busch, P.; *Ärztliche Kosmetologie* **19** 270-315, 1989

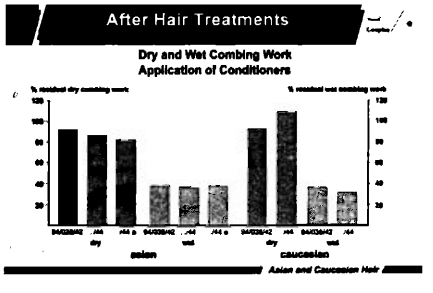


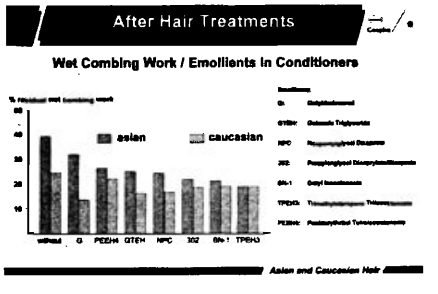












THERMAL DEGREDATION AND PROTECTION OF HAIR

Roger L. McMullen, Jr. and Janusz Jachowicz, Ph. D.
International Specialty Products, Wayne, NJ 07470

Introduction

The literature reflects a limited amount of research focusing on the irreversible chemical and physico chemical changes occurring in hair as a result of thermal treatment, which is applied to hair in conjunction with the use of curling irons or hair-dryers, i.e. in the temperature range from 100 °C to 170 °C [1-5]. The present study concentrates on the effects of thermal treatments on human hair induced by conventional curling irons, operating in the temperature range from 130 °C to 164 °C. The extent of hair degradation was probed by using a tryptophan assay, Hunter colorimetry, texture analysis, and mechanical combing measurements for detecting changes in the inter-fiber frictional properties. The thermal protection of hair against structural damage was investigated by employing PVP/DMAPA Acrylates Copolymer and Quaternium 70.

Materials & Methods

In order to examine the thermal effects on different types of hair, several experiments were performed on bleached and Piedmont hair purchased from DeMeo Brothers, Inc., as well as Asian, light brown, and unpigmented (white) hair from International Hair Importers, Inc. Hair samples, in the form of 6.5" x 1.25" tresses, were precleaned with 3% w/w ammonium lauryl sulfate solution and thoroughly rinsed prior to experimentation. Thermal treatment of hair was performed using a commercial curling iron, operating in the temperature range from 130 °C to 165 °C. In order to maintain uniformity of the experimental conditions and to assure reproducibility of the obtained data, the thermal treatment to each hair tress was administered in the same position. For thermal protection, the hair tress treatments were administered with 1% solutions of the indicated active in which 3.46 mg of active was applied per each gram of hair. The treated tress was then air-dried (23 °C) with an Elchim Professional hair dryer (Model EC 35227) distributed by Elchim – USA. The treatments described above (PVP/DMAPA Acrylates Copolymer and Quaternium 70) are commercial products sold under the trade names of Styleze CC-10 (ISP) and Ceraphyl 70 (ISP), respectively. Fluorescence measurements were performed using a Fluorolog-2 spectrophotometer (Spex Industries) equipped with a fiber-optic sample accessory. The experimental procedures were similar to those described in reference [6]. The combing measurements of hair tresses were performed by employing a Diastron Miniature Tensile Tester operated by MTTWIN software. In order to quantify the degree of color changes resulting from heat treatment in various types of hair, we used a HunterLab ColorQUEST Sphere Spectrocolorimeter manufactured by Hunter Associates Laboratory, Inc., Reston, VA, USA. The use of the spectrocolorimeter enables us to obtain the tristimulus (L, a, b) values, which were utilized to calculate discoloration parameters as a result of thermal treatment.

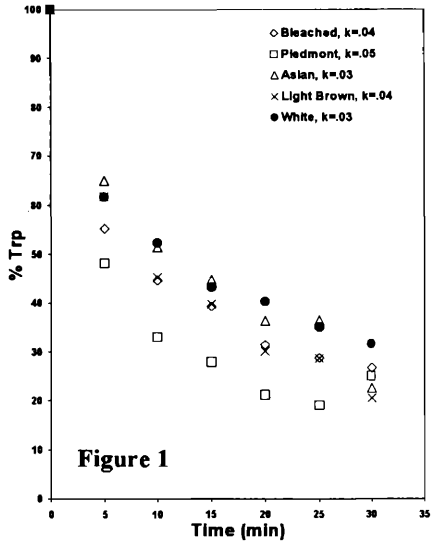
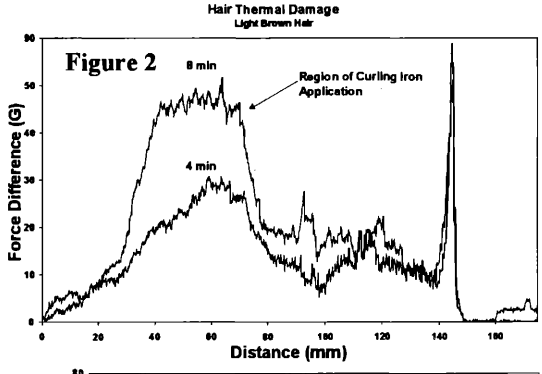
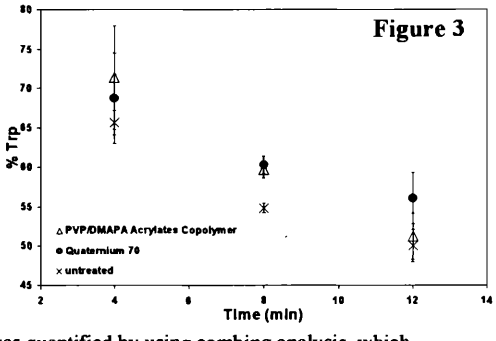


Figure 1

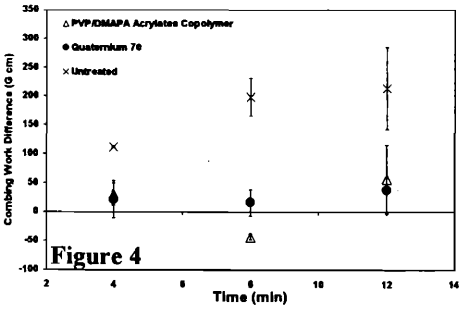
Results & Discussion

The effects of thermal treatments on human hair induced by conventional curling irons, operating in the temperature range from 130°C to 164°C, have been investigated. The fibers were thermally exposed by continuous heating for extended periods of time (3-15 min) or by short (15 seconds) intermittent heating cycles. The model calculations of heat transfer through a fibrous assembly, based on heat





conduction through a semi-infinite solid, were performed. The calculated data have shown that near-uniform temperature distributions are reached in the hair samples within a few seconds of thermal exposure, suggesting that continuous and intermittent modes of treatment are equivalent. The resulting damage to the fibers has been investigated and quantified by the use of fluorescence spectrophotometry, Hunter colorimetry, and combing analysis. The fluorescence analysis analysis has shown that thermal treatment results in a decomposition of hair chromophores, specifically tryptophan (Trp) and its oxidation products (kynurenines). The calculated first-order rate coefficients of Trp decomposition were in the range from 0.03 to 0.12 (min^{-1}) with an estimated activation energy of 6.6 kcal/mol. Figure 1 illustrates the decomposition of Trp as a function of the thermal treatment time for various types of hair at 164 °C. At this temperature, the extent of Trp decomposition is very high reaching nearly 80% conversion after 30 minutes. In addition to this, we have employed Hunter colorimetry to quantify the thermally induced color changes in hair, such as an increase in the yellowness of white and Piedmont hair, or simultaneous yellowing and darkening of bleached hair. Surface damage, resulting from thermal treatment, was quantified by using combing analysis, which revealed a gradual increase in combing forces that were measured in the tress section exposed to the curling iron. Figure 2 provides a representative example of the combing force increases resulting from thermal exposure at 152 °C for light brown hair. Force difference curves were determined by subtracting the curve obtained after thermal degradation from the curve obtained prior to any thermal treatments. A possible method for preventing thermal damage to hair has been tested by pre-treating fibers with PVP/DMAPA Acrylates Copolymer and Quaternium 70. The decomposition of Trp in fibers pretreated with the indicated actives, followed by thermal exposure, was monitored. Figure 3 presents the Trp degradation at 152 °C as a function of the thermal treatment time for light brown hair. As indicated by the data in Figure 3, depositing a layer of surfactant on the surface of hair appears to provide some degree of Trp protection. We also monitored the surface modifications of the fibers, resulting from thermal treatment at 152 °C, by combing analysis. Figure 4 portrays combing work difference as a function of time for hair treated with the indicated actives and for untreated hair. The combing work difference values were determined by integrating the area of the combing curve where thermal treatment was administered and subtracting this value from the similarly obtained value prior to thermal exposure. Similar studies were conducted at 132 °C and provided comparable data with respect to the possible thermal protection properties of the tested compounds.



References

[1] P. Milczarek, M. Zielinski, and M. Garcia, *Colloid Polym. Sci.*, **270**, 1106 (1992).
[2] R. Crawford, C. Robbins, and K. Chesney, *J. Soc.Cosmet. Chem.*, **32**, 27 (1981).
[3] L. Rebenfeld, H. Weigmann, and C. Dansizer, *J. Soc. Cosmet. Chem.*, **17**, 525 (1966).
[4] W. Humphries, D. Miller, and R. Wildnauer, *J. Soc. Cosmet. Chem.*, **23**, 359 (1972).
[5] R. Arnaud, G. Perbet, A. DeFlandre, and G. Lang, *Int. J. Cosmet. Sci.*, **6**, 71 (1984).
[6] C. Pande and J. Jachowicz, *J. Soc.Cosmet. Chem.*, **44**, 109 (1993).

THE ISOLATED PERFUSED BOVINE UDDER SKIN (BUS) MODEL: SURFACTANTS/SKIN INTERACTIONS AFTER SHORT AND LONG TERM EXPOSURE

W. Pitterman[1], J. Kahre[1], H. - U. Krächter[2], Th. Förster[1], L. Kintrup[1] and M. Kietzmann[3]

[1]Henkel KGaA, D-40191 Düsseldorf, Germany; [2]Henkel Corp., Ambler, PA 19002;

[3]Institute for Pharmacology, Pharmacy and Toxicology, Vet. School Hannover, D-30559, Hannover, Germany

INTRODUCTION:

The isolated perfused bovine udder skin model (1) was originally introduced as an *in vitro* model to study the percutaneous absorption. Under *in vitro* conditions barrier properties and metabolization comparable to living skin are maintained in perfused skin models. Due to this viable status additional information concerning time-dependent skin irritation can be obtained in punched skin biopsies representing epidermal and dermal layers. The model gives the opportunity to distinguish cytotoxicity (modified methyl tetrazolium assay) (2) from irritancy (prostaglandin E_2-tissue concentration). The period of exposure (0.5 hour up to 5.0 hours) as well as the type of application (open / occluded; Finn Chamber R) is variable within the perfusion time of approx. 8 hours.

EXPERIMENTAL:

Surfactants: Alkyl polyglycoside [APG] (pH.: 5.5; 3 % and 10 % active substance);
Sodium Lauryl Sulfate [SLS] (pH.: 5.5; 3 % and 10 % active substance);
The unpreserved surfactants were handled as frozen samples during the logistic procedures before the application. The surfactants were applied occlusively using a chamber (D=18 mm; 500µl / Finn Chamber ®) under adhesive tape.

Model: The test set-up is extensively described (1). The viability of the perfused udder was demonstrated by a nearly unchanged glucose consumption, an initially decreasing and thereafter unchanged lactate production and an unchanged dehydrogenase activity in the perfusate after it has flowed through the vascular system. It may be assumed that no significant edema developed within 6 hours since the skin fold thickness was constant.

Morphology: Samples of the treated and untreated skin underwent a routine preparation for H&E histology and transmission electron microscopy.

Biochemistry: *Cytotoxicity:* The MTT-assay indicates the level of impaired mitochondria in the cells of the epidermal layers and dermal tissue. The modified MTT (methyl tetrazolium salt dye conversion, µg Formazan / µg DNA) is used (Maaß, 1993). *Irritancy:* The results of the assessment of the prostaglandin E_2-concentration (ng /µg DNA) in the epidermal and dermal layers presents the level of a mediator substance responsible for erythema and edema formation in the case of clinically evident skin irritation (Maaß, 1993).
For both assays the whole skin biopsy (D=6mm, approx. 4 mm depth) is used. Due to the different cell concentrations in the epidermal and dermal layers, the tissue preparations were adjusted to the comparable DNA content before being analyzed. For the statistical analysis the t-test was used.

CONCLUSION:

Cytotoxicity *(MTT, Figure 1):* After an occlusive exposure period of 1 hour the application of APG 3 % and 10 % AS does not induce a statistically significant cytotoxic potential compared to the untreated skin area whereas the application of SLS 3 % and 10 % resulted in a statistically significant difference ($p < 0.05$). After 1 hour of exposure the cytotoxicity may be predominantely influenced by physicochemical processes which in the case of SLS was much more pronounced than when applying APG.
After an exposure period of five hours the cytotoxic potential shows a comparable level ($p < 0.05$). This demonstrates that the exaggerated test conditions allow a skin penetrating effects of the surfactants which is much less pronounced in the case of APG than when using SLS. APG 10 % AS has about the same potential as APG 3 %or SLS 3 % AS. SLS 10 % AS after a period of exposure of 1 hour and 5 hours impairs about 30 % and 50 % resp. of the epidermal and dermal cells.

Irritancy *(PGE_2-synthesis, Figure 2):* After one and five hours exposure period there was à statistically significant difference ($p < 0.05$) for APG 3 % AS and APG 10 % AS compared to the untreated control. Again no real difference could be observed between APG 3 % and APG 10 % AS .

Morphology: The routine histology did not reveal major alterations in the structure and morphology of the stratum corneum induced by SLS after an exposure period of one hour. The transmission electron microscopy evidences after the prolonged occlusive exposure period of five hours swollen stratum corneum lamellae and small focal clefts between the lamellae.

The pathogenesis of *cytotoxicity* in the skin is a quite different process from *irritancy* and is not linked up closely. The MTT- and PGE_2-levels of SLS 10 % and 3 % AS at 1 hour and 5 hours of exposure differ markedly, wheras with APG, the values are rather close, thereby disclosing a higher skin compatibility of the latter up to 10 % AS. The difference between both surfactants regarding skin compatibility may also be explained with a remarkable low cytotoxic potential of APG after 1 hour of exposure which means almost no physicochemical effects, combined with a low influence on the synthesis of mediator substances.

FIGURE 1

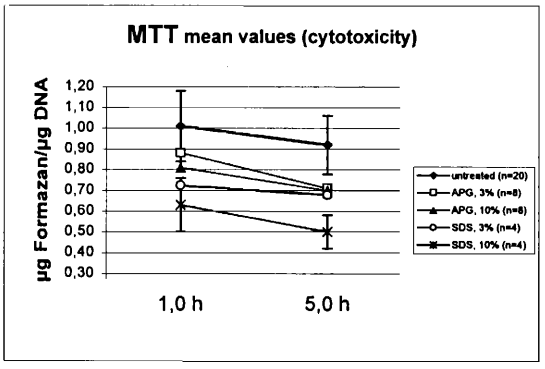
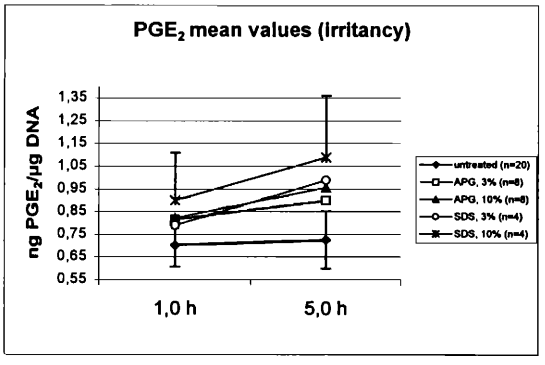


FIGURE 2



(1) Kietzmann, M. et. al. J. Pharmacol. Toxicol. Meth. 30. (1993) pp 75-84
(2) Pittermann, W. et al. In Vitro Toxicology 10 (1997) pp 17-21
(3) Maaß, P. Das isoliert perfundierte Rindereuter-Ein Modell zur Prüfung der Hautirritation ? (Thesis) Tierärztliche Hochschule Hannover (1993)

EVALUATING THE MILDNESS/IRRITATION POTENTIAL OF SURFACTANTS IN PERSONAL CARE PRODUCTS

Al Gabbianelli, Robert Hillermeier, Martha Tate and Michael Prendergast

Rhodia, Inc., Cranbury, NJ 08512

Surfactants and surfactant blends are used in virtually every aspect of modern cosmetic chemistry. Their use as wetting and spreadability additives, as emulsifiers for creams and lotions, and as active ingredients in hair care, skin care and oral care preparations are wide spread and well known. Exposure of the general public to these materials on a regular basis has therefore become virtually inevitable. As a consequence, surfactants and surfactant blends must elicit negligible levels of irritation and meet increasingly higher standards for product mildness.

While surfactants are routinely subjected to a wide battery of safety and irritation assessments there has historically been a paucity of published studies on the irritation potential of surfactants in humans. This presentation will focus on recent studies used to evaluate the irritation potential of several key surfactants that are currently of interest in the personal care arena. Case studies will be reviewed in which various instrumental, clinical and subjective evaluations were used to assess both the beneficial and adverse effects of these materials including the determination of mildness and irritation potential.

Simple irritation (versus sensitization) is generally classified into two broad categories: irritation resulting from a single exposure and irritation resulting from multiple, repeated or extended exposures. As a result of the aversion to animal testing in recent years and the difficulties associated with the interpreting and correlating in-vitro techniques to human responses, clinical patch testing remains the most predictive and widely accepted technique used to characterize both types of irritation. Forty eight hour patch tests are used to assess irritation resulting from a single protracted exposure while 14 or 21 day cumulative patch testing is used to determine the effect of repeated and extended exposure. Specialized evaluation procedures such as the forearm flex wash or modified forearm flex wash have also become popular for the evaluation of irritation potential.

Consequently, the 48 hour patch test was initially used to evaluate the irritation profile of Sodium Laureth Sulfate (SLES), Sodium Cocoamphoacetate (SCA) and combinations thereof. All surfactants and surfactant combinations were evaluated as 3.0% (w/w) aqueous solutions. Results indicated that the greatest irritation is elicited by the pure anionic surfactant SLES and that irritation potential decreases proportionally upon addition of the amphoteric surfactant SCA (Figs. 1 & 2).

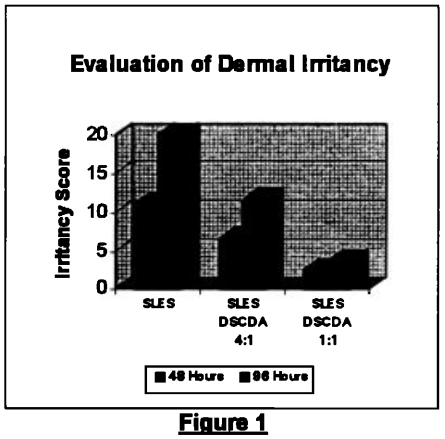


Figure 1

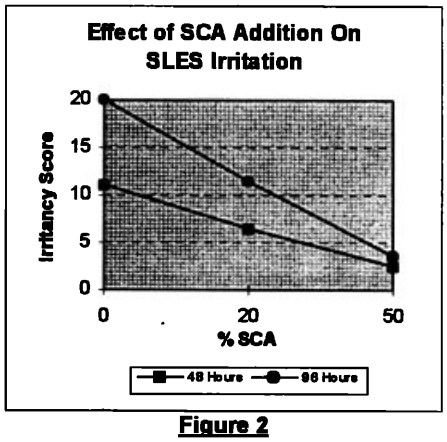


Figure 2

Based on the results of the 48 hour patch test, a 14 day cumulative irritation study was initiated in order to develop a more comprehensive portfolio on surfactant irritation profiles in humans. The study was expanded to include: Sodium Lauryl Sulfate (SLS - as a positive control), SLES, the newer high purity version of Sodium Cocoamphoacetate (SCA) and Potassium Mono Alkyl (C_{12}) Phosphate (MAPS). In addition to the subjective evaluation of irritation by professional clinicians, Corneometer and Chromameter measurements were used to respectively measure surfactant associated dryness and to instrumentally quantify and correlate reddening with clinical erythema scores.

To insure that all materials were evaluated at equivalent levels each of the surfactants listed above was diluted with DI water to a concentration of 0.5% active material. Surfactant blends were prepared by mixing appropriate volumes of the diluted materials. For example, 100 ml of a 0.5% (w/w) surfactant solution containing 75% SCA dilution and 25% of SLES dilution was prepared by mixing 75 ml of 0.5% SCA with 25 ml of 0.5% SLES. In addition, the 0.5% stock dilution of each respective surfactant was used to determine the irritation potential of the pure unblended surfactants.

Results of the 14 day cumulative irritation test indicated that in all cases the pure MAPS and Amphoteric surfactants elicited lower irritation profiles than the comparable surfactant/SLES combinations or the pure SLES sample. Irritancy responses (as determined by the Mean Cumulative Irritation Score) for the pure surfactants indicated that MAPS was the least irritating followed closely by Cocoamphoacetate while SLES and SLS were the most irritating. Irritancy responses for the pure surfactants were ranked as follows:

Least Mild – SLS < SLES < SCA < MAPS -- **Most Mild**
Mean Irritation Score – 40.3 | 30.9 | 11.8 | 4.48

As in the original forty eight hour patch test, combinations of SLES with amphoteric concentrations above 25% were found to attenuate the irritancy potential of SLES with irritancy reductions being roughly proportional to the level of added amphoteric. Irritancy potentials of the anionic:amphoteric combinations at a ratio of 3:1, were not different from those observed for pure SLES. Results obtained for Corneometer and Chromameter measurements were in general agreement with the subjective expert evaluations. Consequently, rankings for surfactant induced dryness and erythema were similar to those obtained for general irritation.

In conclusion, results obtained by the 48 hour patch test for SCA / SLES were predictive of results generated by the 14 day cumulative irritation test. In addition, good agreement was found between the subjective and instrumental techniques. These findings confirm the mildness of MAPS and amphoterics in human clinical studies and demonstrate their ability to enhance the mildness of mixed surfactant systems. Additional testing will be required to evaluate the irritation potential of other neat and mixed surfactant systems.

SURFACTANT ASSOCIATION STRUCTURES AND PHASE CHANGES DURING EVAPORATION OF COSMETIC EMULSIONS

[1]P. Aikens, [2]S.E. Friberg, [2]T. Huang, [2]Y. Yang, [2]H. Yang

[1]ICI Surfactants, Wilmington, DE 19850; [2]Dept. of Chemistry, Clarkson University, Potsdam, NY 13699

INTRODUCTION:

The changes which occur due to evaporation in a cosmetic emulsion after application to the skin can have a significant impact on the performance and efficacy of the product. In a straightforward water/non-volitile oil/surfactant system, the evaporation of water increases the relative concentrations of oil and surfactant, causing surfactant association structures such as hexagonal or lamellar liquid crystalline phases to appear. The path taken follows a straight line from the 100% water corner of the ternary phase diagram through the formulation point (Figure 1)[1] if the oil has a high vapor pressure. If the oil is volatile, a more complex curved path from the water corner results. Figure 2 shows the exact phase changes in the W/O emulsion at various points marked along the evaporation path. Implications from a skin-care point of view are that the formulation could pass through or end up in a 1 ,2 or 3-phase region during the evaporation process, depending on initial composition and also that chemical potential of actives can be controlled[2]. The phase behavior of water, vegetable oil, nonionic surfactant and the sunscreen active octyl dimethyl p-amino benzoic acid (ODP)[3] is examined as well as that of the humectant polymer sodium hyaluronate in water and surfactant[4].

EXPERIMENTAL METHODS:

Phase Diagrams: Maximum solubility boundaries were determined visually after titration with oil or water. Liquid crystalline phases were identified and differentiated by patterns produced under cross-polarized optical microscopy and also by low angle X-ray diffraction.

Emulsion Preparation: Water and polysorbate-80 are mixed and soy oil added with thorough mixing. Centrifugation of the W/O emulsion separated the oil and aqueous phases which were used as described below.

Evaporation Studies: Samples of water, fragrance oil, surfactant were stirred in a Petrii dish at 22°C and weight monitored. At certain points along the evaporation path, determined by weight loss of water, samples were subjected to gc analysis to determine vapor pressure. For the microscopic observation studies of W/O emulsions, a small droplet of oil was placed on a microscope slide and aqueous solution introduced with a syringe and the changing phase behavior observed under crossed polarized microscopy and weight changes monitored. For O/W emulsions, the emulsion is applied to a confined area of a slide and visually observed under the microscope during evaporation.

RESULTS AND DISCUSSION:

Liquid crystalline phases can be formed in even simple systems such as seen in Figure 1. Surfactant association structures are formed from water and surfactant. A micellar solution is formed up to 40% surfactant (in equilibrium with oil) after which a hexagonal liquid crystalline phase appears. This is in equilibrium with oil and an aqueous solution with maximum surfactant. Another triangular 3-phase region is seen with the hexagonal liquid crystal in equilibrium with the oil and a surfactant-oil (L_2) solution. Evaporation paths for the W/O and O/W emulsions are traced with a dashed line.

The W/O emulsion of water, soy oil and polysorbate-80 between crossed polarizers under the optical microscope is shown Figure 2. Initially, the view is black for the oil and aqueous isotropic liquid phases. Photo 1 shows a thin radiant band which appeared as the liquid hexagonal crystalline phase appears at point b on the phase diagram in Figure 1. This birefringent area grew (photo 2) to cover the entire droplet, seen in photo 3. Photo 4 shows the liquid crystalline phase diminishing as it is replaced by isotropic solution the droplet again appears black at the end (photo not shown).

Figure 3 shows an example of the phase behavior of the sunscreen active ODP in water and laureth 4. The evaporation paths of an initial 2-phase (water and lamellar liquid crystal) and 3-phase (water, laureth 4-ODP solution and lamellar liquid crystal) formulation are traced by the dashed lines. Again, the various phases can be seen by polarized optical microscopy at points along the water evaporation path. The location of ODP in the lamellar liquid crystalline phase was determined by low angle X-ray diffraction and by UV spectroscopy to be in the B region of the bilayer, shown in Figure 4 while the vegetable oil was distributed between regions B and C.

Another interesting system which was examined was that of the moisturizing polymer hyaluronic acid (in sodium salt) a and water in the presence of either polysorbate 80, (which gave a hexagonal liquid crystal) or a phospholipid/fatty acid emulsifier (giving lamellar liquid crystals). As water evaporated from the laureth 4 system, polarized light microscopy showed the appearance of lyotropic nematic liquid crystalline structures. This could not be reproduced by mixing/heating the components together in the ratio at which the structure appeared, it only could be formed during the slow evaporation process.

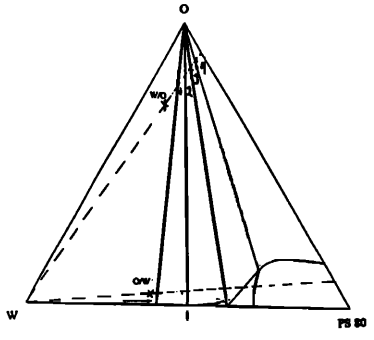


Figure 1. Phase diagram of Soy Oil, Polysorbate 80, Water.
—— Water Evaporation Paths for W/O and O/W Emulsions

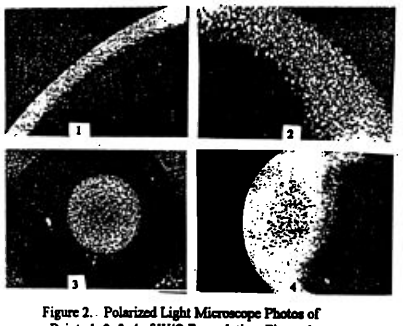


Figure 2. Polarized Light Microscope Photos of Points 1, 2, 3, 4 of W/O Formulation, Figure 1

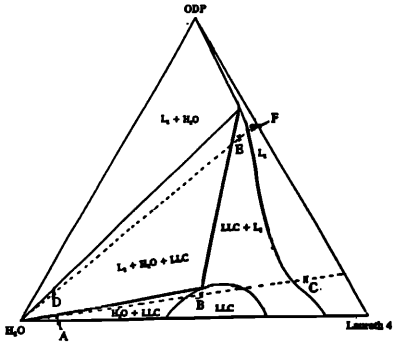


Figure 3. Phase Diagram of ODP, Laureth 4, Water
—— Evaporation Paths for 2-Phase and 3-Phase Emulsions

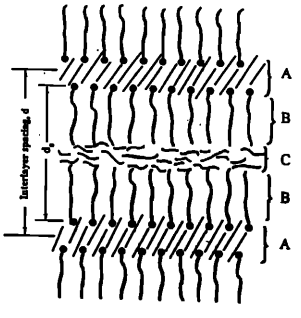


Figure 4.Model of Lamellar Liquid Crystal Bilayer
d = Interlayer Spacing, d0 =Zero Water Spacing (from X-ray)
A = Aqueous B = Amphiphilic Layer, C = Non-polar Region

REFERENCES:

1.) Friberg, S.E., Huang, T., Aikens, P. A., *Colloids Surfaces A: Physiochem Eng. Aspects*, **121**, 1, (1977)
2.) Friberg, S. E., Brin, A-J., *J. Soc. Cosmet. Chem.*, **46**, 255, (1995)
3.) Friberg, S. E., Yang, J., Yang, H., Aikens, P. A., *J. Am. Oil Chem. Soc.*, in press
4.) Friberg, S. E., Yang, H., Aikens, P.A., submitted for publication

TRANSIENT NETWORK COPOLYMERS – SURFACE PROPERTIES AND ASPECTS OF SKIN INTERACTIONS

Stephen M. Greenberg, Ph.D., Alex L. Vainshelboim, Ph.D., Alena Lovy and Michael Esposito

Lipo Chemicals, Inc., Paterson, NJ and HYMEDIX International, Dayton, NJ

INTRODUCTION

Hydrogels are found in almost every aspect of the human experience. They are used in various industrial, medical and scientific applications (latex paints, wound care and cultivation media are examples), in foods (think of the *Jello* you recently ate), and of course, in cosmetics and personal care products. This paper will concern itself with this latter industry; that is, the chemistry, characterization and application of a unique and unusual polymeric materials (produced through the controlled base-catalyzed hydrolysis of polyacrylonitrile)[1], in cosmetic and personal care products, particularly as related to the surface modification of skin.

Hydrogels can be prepared from either natural polymers such as gelatin or polysaccarides, or from synthetic polymers such as polyacrylic acid or polyacrylonitrile derivatives. In either group is possible to modify the polymer to literally design the properties of the final hydrogel. This capability has created a broad variety of polymers (and resultant hydrogels) that vary just as broadly in their properties. Polymers that can form hydrogels will have the ability to hold water (from low swelling to superabsorbents), to degrade biologically (from none to complete), and may exist as either of the two basic physiochemical categories (thermosetting or thermoplastic.) For the purposes of this discussion, thermosetting polymers are defined as being covalently crosslinked during synthesis, cannot be reshaped once set, and may form permanent network hydrogels. Likewise, thermoplastic polymers are defined as not being covalently crosslinked, and form may transient network hydrogel. Additionally, the subject polymer of this paper produces thermoplastic hydrogels that also display non-Newton rheology (thixotropy.) This combination of unusual and unique properties results in hydrogels beneficial for cosmetic and personal care application.

[1]-U.S. Patent Number 4,943,618

PROPERTIES AND APPLICATIONS

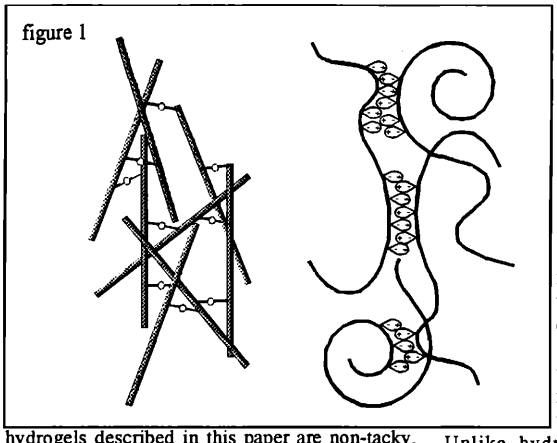
Through the process of controlled base-catalyzed hydrolysis, polyacrylonitrile is transformed into polymers capable of producing hydrogels distinctly different from other natural and synthetic polymers intended for skin care applications. Original technology allows the creation of medium molecular weight polymeric structures that contain about 20% hydrophilic groups segmented within the primarily hydrophobic character of the chain. It is this arrangement of hydrophilic and hydrophobic segments that gives the hydrogels their unique and desirable qualities; that is, this arrangement, in the aqueous environment, allows the independent polymer molecules to electrostatically interact and form non-covalent, transient bonds. This unique interaction allows the creation of matrices that can deform and reform during energy input without destroying the polymer. The result is hydrogels having thixotropic rheology that form thin, uniform and non-tacky films. In addition, the polymer will orient on the skin due to interactions between the hydrophilic skin surface and the hydrophilic moieties in the polymer. The result of this orientation is to present to the environment the hydrophobic portions of the molecule, interpreted as 'silky' to the touch.

Figure 1 schematically represents the essential differences in structure between polymers that produce permanent network hydrogels (left) and those that produce transient network hydrogels (right).

Hydrogels serve the primary purposes in skin care formulations to improve viscosity and to aid in the delivery of active ingredients. Traditional (thermosetting/permanent network) hydrogels suffer from a number of drawbacks in these respects; i.e., tackiness, non-uniformity of film thickness, are irreversibly shear-thinned, are sensitive to electrolytes and pH extremes, and make little, if any, positive contribution to after-feel. On the other hand, the novel (thermoplastic/transient network)

TRANSIENT NETWORK COPOLYMERS – SURFACE PROPERTIES AND ASPECTS OF SKIN INTERACTIONS
Cont.

figure 1



hydrogels described in this paper are non-tacky, leave uniformly thin films on the skin, are reversibly shear-thinned (thixotropic), tolerate electrolytes and pH extremes very well, and confer a 'silky', low friction feel.

The ability of the transient network hydrogels to deform and reform accounts for several of their unique and desirable properties. Lack of tackiness in these systems is a result of the structural flexibility (non-covalent bonding) of the hydrogel's matrix, as is the ease of spreading. The thixotropic character of the hydrogel matrix, likewise, enhances uniformity of film thickness and the resultant distribution of active ingredients.

The topic polymers of this paper orient so that the hydrophilic portions are in contact with the skin and the hydrophobic portions form a protective layer above. This not only accounts for the pleasant after-feel, but also produces a protective barrier that reduces TEWL. The segmented hydrophilic-hydrophobic segmentation of these polymers also gives them the ability to cross the oil-water interface and assist in emulsion stabilization.

CONCLUSIONS

Transient network hydrogels formed from non-covalently crosslinked polymers offer many significant advantages over traditional hydrogels. Our work, based on the development of controlled base-catalyzed polyacrylonitrile polymers, has shown that hydrogels derived from these polymers display thixotropic and non-tacky properties, cast uniform, continuous films and assist in the stabilization of emulsions typical of skin-care formulations. The presentation of hydrophobic moieties to the environment confers a smooth feel to the skin and provides a protective barrier that reduces TEWL. Unlike hydrogels derived from covalently crosslinked polymers, the subject hydrogels are unusually resistant to electrolyte level and pH extremes.

All of these properties, as well as numerous others, are very beneficial to meet the emerging demands of the skin-care market.

The Clinical and Laboratory Assessment of Skin Whitening

Walter Smith, Ph.D. Dermac Laboratory, Stamford, CT
and
Scott Norton, Ph.D., University of Texas, Denton

Skin whitening can be evaluated with a variety of in vitro and in vivo test methods with duel goals of optimizing product performance and providing substantiation for product claims.

Test tube assays monitoring the tyrosinase dependent conversion of tyrosine to L-Dopa have been useful in comparing the activity of tyrosinase inhibitors. Our tests indicate that kojic acid, ascorbyl magnesium phosphate, Mulberry extract, and numerous herbal extracts are effective enzyme inhibitors. This in vitro activity however does not always translate into clinical effectiveness. In a different test system we have evaluated the inhibition of delayed hyper-pigmentation resulting from complete removal of the stratum corneum via tape stripping. This in vivo test has confirmed activity of kojic acid, and some Mulburry extracts, however vehicle differences could markedly influence activity, and correlation with the in vitro assay was poor.

Finally we have evaluated the skin whitening effects of several cosmetic prototypes over a three to four month period. Clinical grading, photography, and the Minolta Meter have proved useful in assessing skin color. Good correlation is observed with the three methods, with clinical grading being most sensitive. We have observed that herbal extracts, hydroquinone, and combinations of AHA's can induce measurable and consumer relevant skin lightening after two to three months of product use. In most cases a general skin lightening is observed without preferential lightening of "age spots".

An In Vitro Method for Screening Whitening Agents

Gopa Majmudar, Ph.D., George Jacob, Yolanda Laboy and Lou Fisher, Ph.D.
Mary Kay Holding Corporation, Dallas, TX 75247

INTRODUCTION:

The development of successful whitening skin care products depends on the use effective whitening or depigmenting ingredients that reduce melanin production in melanocytes. We utilized a three dimensional human skin model, Melanoderm, to screen whitening agents before testing the product on humans.
Melanoderm is a living skin equivalent *in vitro* model of the human epidermis consists of well differentiated human keratinocytes and melanocytes. Biochemical, histological and ultrastructural properties of Melanoderm are similar to human epidermis. The cross section of the Melanoderm shows the presence of stratum corneum, keratinocytes and dendritic melanocytes localized in the basal cell layer. Melanocytes stain positive when exposed to L-dihydroxyphenyl alanine (L-DOPA), a precursor of melanin. A relative activity of whitening agents such as kojic acid, lactic acid and magnesium ascorbyl phosphate (MAP) was measured on Melanoderm. Total kojic acid in the product was measured by high performance liquid chromatography (HPLC). A combination of *in vitro* test on human skin equivalent culture and HPLC method is useful to evaluate the efficacy, stability and toxicity of whitening ingredients and products before clinical testing. Finally, the product was tested on human subjects to obtain a better correlation with *in vitro* test results.

MATERIALS AND METHODS:

In Vitro Testing: Melanoderm was purchased from Mat Tek Co, Ashland, MA. Kojic acid, lactic acid and MAP in aqueous or anhydrous base and the base alone were applied to Melanoderm and incubated for two to three days. At the end of incubation, the cream was removed . Melanoderm was washed in phosphate buffer saline (PBS) and incubated in 0.1% L-DOPA for one hour at room temperature. After one hour Melanoderm was placed in 0.1% fresh L-DOPA and incubated 4 to 16 hours. Optical density was measured at 490nm using a microplate reader.
HPLC : Kojic acid assay was performed using a HP laser jet system with a four plus integrator. A C_8, ODS -5, 150 X 4.5 mm column (Metachem #0297) and a mobile phase composed of water; acetonitrile (20:80 v/v) were used. The wavelength of detection was 254nm. Samples were diluted with water:acetonitrile (50:50 v/v).

Clinical Testing: Ten healthy adult subjects, who gave informed consent, participated in a 12 week study. All subjects applied anhydrous and aqueous base products containing kojic acid and a retail product containing hydroquinone on their forearms twice daily. Baseline and 24 hours later, chromometer measurements were taken at each of the test sites.

RESULTS AND DISCUSSION:

Kojic acid (1%), lactic acid (3%) and 1% MAP in nonionic aqueous base were applied for three days on Mclanoderm. Melanoderm was treated with L -DOPA as described in Materials and Methods and examined under the microscope. The treated Melanoderm looked dendritic and healthy, suggesting that whitening agents and a base were not toxic. A quantitative analysis (Table-1) showed 48% and 46% and 33% inhibition by kojic acid, lactic acid and MAP respectively.

To confirm our results and check the stability of kojic acid in different bases, we developed a new HPLC method to measure kojic acid in different products. Surprisingly, HPLC analysis showed that kojic acid in aqueous base was not stable (Table-2). Based on these data, we developed a new anhydrous base and tested on Melanoderm with kojic and lactic acids (Table -1). HPLC data suggested that kojic acid was stable in the anhydrous base (Table -2).

Table 1 Effect of whitening ingredients on Melanoderm

Ingredients	Concentrations (%)	Tyrosinase Inhibition (%)	
		Aqueous Base	Anhydrous Base
None	0.00	0.00	0.00
Kojic acid	1.00	47.98	41.00
Lactic acid	3.00	46.20	22.00
MAP	1.00	33.55	ND

Table 2 HPLC analysis of kojic acid

Base /Vehicle	Time (weeks)	Kojic acid concentration (%)			
		25° C (%)	Lost (%)	37° C (%)	Lost (%)
Aqueous	0.00	1.15	0.00	ND	ND
	2.00	1.23	0.00	0.98	14.79
	5.00	0.14	87.83	0.18	86.35
Anhydrous	0.00	0.99	0.00	ND	ND
	8.00	ND	ND	0.78	21.22
	12.00	ND	ND	0.80	19.20
	26.0	0.97	2.03	ND	ND

To confirm our *in vitro* data, clinical testing was conducted with anhydrous and aqueous nonionic bases containing kojic acid. There was a gradual increase in lightening of the skin over a period of three months with the anhydrous base containing kojic acid (Table -3). Non-ionic aqueous base containing kojic acid was less effective (Table -3), probably due to loss of kojic acid over a period of three months. Thus, we obtained a correlation among *in vitro*, analytical (HPLC) and clinical data on whitening /lightening agents.

Table 3 Clinical study on skin whitening by chromometer

Products	Change from no treatment (%)					
Kojic acid	1 Month	p value	2 Months	p value	3 Months	p value
Aqueous base	0.00	0.95	0.9	**0.029**	0.2	0.73
Anhydrous base	0.80	0.09	1.6	**0.006**	2.6	**.0003**
Hydroquinine product	1.10	**0.05**	0.2	0.61	2.8	**0.019**

Bold: Significant p values.

Patent pending. * Y. L. worked at Mary Kay Holding Co.

A Sensitive and Quantitative Split-Face Clinical Protocol to Discriminate Cosmetic Formulations for Facial Hyperigmentation Reduction Efficacy

Kukizo Miyamoto and Greg G. Hillebrand

Procter & Gamble Far East, Inc., Kobe, Japan

INTRODUCTION:

Facial skin hyperpigmentation, e.g., senile lentigines, freckles, post-inflammatory hyperpigmentation, and melasma, is a problem for all peoples of the world but is particularly problematic for Asians[1,2]. Sound clinical data that unequivocally proves the visible skin benefits afforded by skin lightening and/or hyperpigmented spot reduction formulations is frequently needed for claims support and regulatory approval. Split-face paired comparison testing has proven to be quite useful in dermatology for treatment efficacy assessment for various skin disorders, especially acne[3]. In this paper, we present results that demonstrate the sensitivity and efficiency of a split-face clinical design combined with high resolution video imaging to discriminate cosmetic formulations for hyperpigmentation reduction efficacy.

CLINICAL METHODS:

Design: Randomized split-face double blind paired comparison study.

Study Period: February-August, 1997.

Subjects: 120 Japanese females, 25-60 years, with moderate to severe hyperpigmentation on both sides of the face.

Test Treatments and Regimen: Subjects (60 per group) were instructed to apply a test formulation (0.26g) to one side of their face and to apply another formulation to the other side twice a day (morning/evening) for 6 months. Test treatment pairs were randomized right/left and were:

	One Side of Face		Other Side of Face
Leg 1	SLM = A commercially marketed Skin Lightening Moisturizer containing a mixture of MHW-approved skin lightening actives (Max Factor Natique CR White)	vs.	NM = A commercially marketed Normal Moisturizer without skin lightening actives (Oil of Olay Sensitive Skin Beauty Fluid)
Leg 2	E = A commercially marketed Essence containing a yeast-conditioned culture medium (SK-II Facial Treatment Repair C)	vs.	V = SK-II Facial Treatment Repair C Vehicle (yeast-conditioned medium is replaced by water)

Skin Measures: Video images of the left and right sides of the subject's clean face were collected at baseline, 1, 3 and 6 months of treatment using a Sony DXC-537H 3CCD color TV camera with a Canon J15x9.5 BKRS lens equipped with a polarizing filter. Facial illumination was provided by two Balcar Flux-Lites equipped with flux tungsten light bulbs (3500^0K) positioned above and below the camera to provide even lighting of the side of the subject's face. Accurate repositioning of the subjects was facilitated by superimposing the live image on the digitally-stored image obtained at baseline. A color chart was used as a standard to calibrate the imaging system each study day. Computer analysis of the video images allowed quantification of basal skin color tone (L*a*b*) and hyperpigmented spot area (mm^2) of pigmented macules.

Reproducibility and Accuracy: The intra-subject coefficient of variance (COV) of L*, a*, and b* values and hyperpigmented spot area for five video images of the same subject (images spaced 10 min. apart) were determined over five subjects. Accuracy was determined using Asian and Caucasian mannequin heads (Figure 1) to which circular artificial spots of known area were applied; the computer image-analyzed area was compared to the true area.

Visual Perception System (VPS): Judges (n=7) viewed each subject's pre-treatment (baseline) vs. post-treatment (1, 3 or 6 month) facial images side by side on a calibrated video monitor (Barco Type 121). The judge was blind to which image was pre/post treatment and the identity of the test treatment. The judges were trained to recognize melanin

hyperpigmentation and to make judgments based on pigmentation differences in the upper cheek and eye area only. The judge indicated which of the two images had less noticeable hyperpigmented spots and rated the magnitude of the difference between images on a 1-4 scale. The magnitude rating was assigned a positive (+) or negative (-) sign depending upon whether (+) or not (-) the judge indicated the post-treatment image had less noticeable spots. Thus, positive values of the magnitude rating indicate spot reduction efficacy and values of zero or less indicate no efficacy.

Statistical Analysis: The difference between the test treatments' (SLM and E) mean change from baseline and their respective controls' (NM and V) mean change from baseline for L*, a*, and b* values and hyperpigmented spot area were compared using a paired T-test (significance set at $p<0.05$). The mean VPS grades for each treatment and control pair were also compared using a paired T-test. A non-parametric statistic analysis (McNemar's test) was used to compare the test products for the number of 'AFTER' images selected as improved in the VPS analysis.

RESULTS:

Reproducibility and Accuracy: The within subject COVs for L*, a*, and b* value measurements in the cheek and eye area were 0.6%, 2.1% and 1.8%, respectively. The within subject COV for the hyperpigmented spot area measurement in the cheek and eye area was 0.5%. The imaging system accuracy for measuring changes in hyperpigmented spot area was determined to be less than +/-5% when measurements were made in the region of the cheek and eye area. All image analysis measurements of live subjects were confined to the area of the cheek and around the eye.

Subject Accountability: Of the 120 subjects enrolled, 104 completed the study (118 completed through 3 months).

Basal Skin Color Tone: There was no significant difference between treatments for L*, a* or b* value change from baseline (see Table I for L*-value change from baseline).

Hyperpigmented Spot Area: Both test treatments (SLM and E) showed significant reduction in hyperpigmented spot area vs. their respective controls at all time points (Figure 2).

Visual Perception System (VPS): The mean VPS grades for the test treatments (SLM & E) were significantly greater than their respective controls at all time points (Figure 3). Judges more often selected the AFTER image (vs. the BEFORE image) for both SLM & E vs. their respective controls (Table II). Figure 4 shows hyperpigmentation improvement on one subject treated with E on one side of her face at baseline and 6 months (image overlays are also shown).

FIGURE 1
Asian Shaped Mannequin Heads

TABLE I
L*-value Change From Baseline

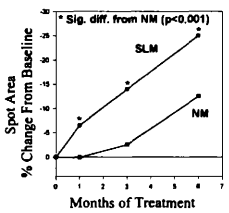
	Months		
Treatment	1	3	6
SLM	-0.27	0.78	0.04
NM	-0.26	0.96	-0.20
E	-0.27	0.61	0.22
V	-0.19	0.55	0.07

TABLE II
VPS % AFTER Images Selected

	Months		
Treatment	1	3	6
SLM	74*	60*	77*
NM	45	37	56
E	60*	43*	62*
V	40	30	43

*Sig. diff. from control ($p<0.025$), McNemar's Test

FIGURE 2
Hyperpigmented Spot Area



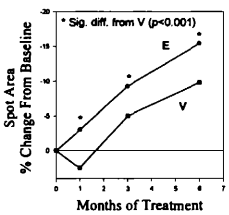
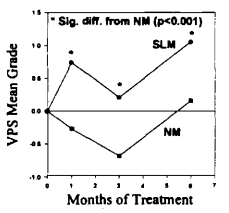


FIGURE 3
VPS Mean Grades



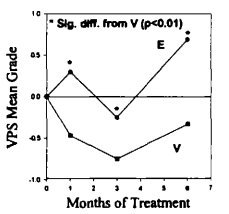


FIGURE 4
Subject Treated With Product E

Baseline image (left) with overlay (right)

6-month image (left) with overlay (right)

CONCLUSIONS:

The split-face protocol design in combination with high resolution video imaging is capable of discriminating the small treatment effects afforded by commercial cosmetic formulations for hyperpigmented spot reduction. Both product formulations tested here significantly reduced facial hyperpigmented spot area after 1 month of use compared to their respective controls as assessed by computer image analysis of video images as well as by visual grading of BEFORE/AFTER image pairs. This benefit was maintained through 6 months of treatment.

REFERENCES:

1. Kang S. and Sober A.J., "Disturbances of Melanin Pigmentation", in *Dermatology*, Moschella and Hurley, Ed., 3rd Ed., **Vol. 2**, 1442-1474 (*1992*).
2. M. Jimbow and K. Jimbow, Pigmentary Disorders in Oriental Skin, *Clinics in Dermatology*, 7, 11-27 (*1989*).
3. Caron D. et al., Split -face Comparison of Adapalene 0.1% Gel and Tretinoin 0.025% Gel in Acne Patients. *J Am Acad Dermatol* **36** (6 Pt 2) S110-112 (*1997*).

NEW POTENTIALS FOR SKIN LIGHTENING

Durant Scholz, Suellen Bennett and Geoff Brooks

Brooks Industries, Inc., So. Plainfield, NJ 07090

We live in a world where everyone wishes to enhance their appearances. For most of the world enhancing appearance involves reducing or evening the pigmentation of the skin.

The largest perceived difference in skin tone is between constitutive pigmentation and adaptive pigmentation. This variation between sun-exposed and unexposed skin is typically about 1000 melanocytes per mm^2. Constitutive pigmentation is the natural level of pigmentation in skin that has not been damaged or exposed to light. It is difficult to reduce the level of pigmentation of an individual below the constitutive level without significantly altering the individual's biochemistry. Considering that melanin blocks or absorbs 90% of the UV radiation penetrating through the stratum corneum, as well as the considerable anti-oxidant potential melanin offers, make the wisdom of looking to alter its constitutive levels questionable.

Adaptive pigmentation is the additional pigmentation due to UV exposure, acne, skin damage, or pregnancy. To truly understand how we can influence adaptive pigmentation and the technology available to do so it is important to first discuss our current understanding of the tanning process. The most important concept is that the difference in pigmentation between light and dark skinned people is not in the number of melanocytes but the mechanism of transfer of melanosomes to the keratinocytes.

Melanin synthesis is an oxidative process. Initial research seemed to show that melanin synthesis was solely controlled by tyrosinase, but now it seems that the process is significantly more complex. Tyrosinase converts tyrosine to dihydroxy phenylalanine (DOPA). Tyrosinase is a copper containing oxidase. DOPA is subsequently converted to dopaquinone (DQ). At this point melanin synthesis can be directed to pheomelanin, the yellow-orange melanin found in blond and red-haired people, or eumelanin, the dark brown melanin found in dark-haired people. For the purposes of this discussion we will confine ourselves to eumelanin production.

Polymerization of dopaquinone produces leucodopachrome. Leucodopachrome is converted to dopachrome which in turn is converted to either 5,6 dihydroxyindole (DHI)

or 5,6 dihydroxyindole-2-carboxylic acid (DHICA). 5,6 dihydroxyindole and 5,6 dihydroxyindole-2--carboxylic acid are oxidized to indole-5,6-quinones, which is in turn further polymerized to form the less well defined melanochrome and finally eumelanin.

Unfortunately for those who are studying the process of melanogenesis, the actual synthesis of melanin is only part of the process. Melanogenesis also involves the development of the melanosomes, the organelle of the melanocyte in which melanin is synthesized, and the subsequent transfer of the melanosomes to keratinocytes.

Melanosome development is generally divided into four phases. In phase I melanosomes contain tyrosinase but are clear. In the second phase the melanosome assumes an oblong shape as microtubules and microfilaments begin to expand. In phase II melanin is being formed. Phase III melanosomes are darker with a higher density and begin to migrate toward the dendrites of the melanocytes. In the final phase the melanosomes are opaque and are ready for transfer from the dendrites to keratinocytes.

Once melanosomes have been transferred to keratinocytes their fate depends extensively on the race of the subject. In Caucasian subjects melanosomes begin to be hydrolyzed almost immediately upon uptake. The degradation continues until no melanosomes are detected by the time that the keratinocytes have migrated into the stratum corneum. In Black skin melanosomes remain intact in the stratum corneum, indicating that there is little or no enzymatic degradation. Melanosomes in Asian skin do degrade but not at the rate seen in Caucasian skin.

To make the subject more interesting all of the aforementioned steps are influenced by environment, genetics, and hormone flux. Haven taken a basic look at the process of melanogenesis, we can now begin to look at ways to prevent or reverse adaptive pigmentation.

There are several routes by which we can slow down melanogenesis. Perhaps the best way to slow down melanogenesis is to reduce the amount of UV light that reaches the skin. If people cannot stay out of the sun, they should attempt to regularly use sunscreens. UV radiation not only directly stimulates the generation of tyrosinase mRNA, but it also provokes the synthesis of Vitamin D_3, which activates tyrosinase, and α-MSH (melanocyte stimulating hormone). As a result of the UV radiation, oxidative stress is generated which in turn generates inflammation. Inflammation, perhaps mediated by cytokines, increases pigmentation.

Perhaps the most talked about means of minimizing adaptive pigmentation is reducing tyrosinase activity. This can be accomplished by blocking the production of tyrosinase, inhibiting the enzymatic activity of tyrosinase, or preventing the uptake of tyrosinase by the melanosomes. Lactic acid and placental extracts suppress tyrosinase production at a genetic level. Materials such as licorice extract, or kojic acid inhibit the enzymatic activity of tyrosinase. Amino sugars such as glucosamine or galactosamine are capable of inhibiting the glycosylation of tyrosinase required for its uptake into melanosomes and subsequent activation.

Another approach to reducing the development of pigmentation is to reduce the activity of melanocytes. Melanocyte activity can be reduced through the use of selectively cytotoxic materials such as hydroquinone. Alternatively materials which are α-MSH antagonists, such as melatonin can be used to prevent the activation of the melanocytes.

The last common approach is to take advantage of the fact that melanogenesis is an oxidative process. Highly reactive antioxidants such as ascorbic acid can be used to compete with melanin precursors for the oxidative stress available to the system. This approach not only inhibits the production of melanin, but can reduce the amount of melanin already present in the skin.

Most commonly used skin-lightening ingredients share a common problem. Most skin-lightening ingredients tend to be unstable in typical cosmetic formulations. Some lighteners such as hydroquinone also demonstrate significant toxicity. In order to maximize the whitening effects while minimizing possible toxicity or formulation incompatibilities it is important to use a multifaceted approach.

The first step in optimizing a skin-lightening formulation is to select actives that will cover several routes of melanogenesis. Initially a broad spectrum sunscreen must be selected. While it may take months to alleviate pigmentation, it may only take one day in the sun to regenerate it. UV filters will also help to improve the stability of any photo-labile actives present. Having selected a sunscreen it is now important to apply a combination of tyrosine inhibitors and antioxidants.

The question remains. How do you take a cocktail of commonly used skin lightening agents and significantly increase the activity? The answer lies in the field of potentiation. By stimulating cell activity it is clearly possible to influence the pigmentation process. Just as cyclic AMP agonists can accelerate tanning, materials that increase cell respiration can reduce melanogenesis.

Many ingredients such as Sacchromyces ferment extract or Clintonia boreallis extract increase cell respiration. These potentiators do not increase the amount of oxygen available to the cell, but increase the efficiency with which the cell utilizes the oxygen. Increase efficiency in oxygen utilization translates to increased efficiency in other process.

Activation of fibroblasts reduces the rate of melanogenesis. Activation of keratinocytes increases the speed at which melanin is eliminated from the skin. Potentiators in conjunction with more traditional approaches to skin lightening offer a revolutionary approach to skin lightening.

The future of research for skin lightening should not focus on looking for new specific lightening agents, but at potentiating the natural processes which can inhibit melanogenesis.

References

1. G. Prota, Melanins and melanogenesis, Academic Press, London 1992.

2. P. Aroca, K. Urabe,T. Kobayashi, K Tsukamoro, and V. J. Hearing, Melanin biosynthesis patterns following hormonal stimulation, J. Biol Chem **268** (34) 25650 (1993)

3. J. R. Jara, F. Solano, J. C. Garcia-Borron, P. Aroca, J. A. Lozano, Regulation of mammalian melanogenesis. II the role of metal cations. Biochim Biophys Acta **1035**: 276-85 (1990)

4. M. T. McEwan, P. G. Parsons, Regulation of tyrosinase expression and activity in human melanoma cells via histamine receptors. J Invest Dermatol **97**: 868-73 (1991)

5. A. K. Chakraborth, S. J. Orlow, JL Bolognia, and JL Pawelek, Structural/functional relationships between internal and external MSH receptors: modulation of expression in Cloudman melanoma cells by UVB radiation, J Cell Physiology **147** 1 (1991)

6. C. Bertolotto, K. Bille, J. P. Ortonne, R. Balloti, Regulation of tyrosinase gene expression by cAMP in B16 melanoma cells involves two CATGTG motifs surrounding the TATA box: implication of the micropthalmia gene product. J. Cell Biol. **134**: 747-755 (1996)

7. P. R. Gordon, C. P. Mansur, and B. A. Gilchrest, Regulation of human melanocyte growth, density, and melanization by keratinocyte-derived factors., J. Invest Dermatol **92** 565 (1989)

8. G. Imokawa, Y. Mishima, Active tyrosinase site in melanin synthesizing cells: Biochemical analysis of coated vesicles, Jap J Dermatol **88** 913 (1978)

9. J. Vachtenheim, J. Duchon, Melanogenic factors: regulation of gene expression. Sb Lek **97**:41-47 (1996)

10. I. Suzuki, R. D. Cone, S. Im, J. Nordlund, Z. A. Abdel-Malek, Binding of melanotropic hormones to the melanocortin receptor MC1R on human melanocytes stimulates proliferation and melanogenesis. Endocrinology **137**: 1627-1633 (1996)

11. G. Imokawa, Y. Yada, M. Kimura, Signaling mechanisms of endothelial-induced mitogenesis and melanogenesis in human melanocytes. Biochem. J. **314**: 305-312 (1996)

12. K. Kameyama, P. M. Montague, V. J. Hearing, The expression of melanocyte-stimulating hormone receptors correlates with mammalian pigmentation and can be modulated by interferons. J Cell Physiol **137**: 35-44 (1988)

13. Signaling mechanisms of endothelin-induced mitogenesis and melanogenesis in human melanocytes. Biochem J. **314**: 305-312 (1996)

14. B. A. Gilchrest, H. Y. Park, M. S. Eller, M. Yaar, Mechanism of ultraviolet light-induced pigmentation. Photochem. Photobiol. **63**: 1-10 (1996)

15. T. Nishimura, T. Komentani, S. Okada, N. Ueno, T. Yamamoto, Inhibitory effects of hydroquinone-alpha-glucoside on melanin synthesis. Yakugaku Zasshi **115**: 626-632 (1995)

16. Y. Mishima, Molecular and biological control of melanogenesis through tyrosine genes and intrinsic and extrinsic regulatory factors. Pigment Cell Res **7495**: 376-387

17. S. Ando, O. Ando, Y. Suemoto, Y. Mishima, Tyrosinase gene transcription and its control by melanogenic inhibitors. J. Invest Dermatol **100**: 150S-155S (1993)

18. Y. Tomita, W. Torinuki, H. Tagami, Stimulation of human melanocyte by vitamin possible mediates skin pigmentation after sun exposure. J. Invest Dermatol. **90**: 882 (1988)

19. P. Valverde, E. Benedito, F. Solano, S. Oaknin, J. A. Lozano, J. C. Garcia-Borron, Melatonin antagonizes alpha-melanocyte-stimulating hormone enhancement of melanogenesis in mouse melanoma cells by blocking the hormone-induced accumulation of the c locus tyrosinase. Eur J Biochem **232**: 257-263 (1995)

A COMPARISON OF SKIN LIGHTENING AGENTS

Evelyn G. Su, Ph.D.

Sino Lion (USA) Ltd., New York, NY 10048

INTRODUCTION:

Skin lightening products have become increasingly popular in the Asian-pacific countries as well as in the African and South American countries. Europe and America have also seen increased interests in skin lightening agents. For Asian and Black skin, the main purpose for skin lightening products is to lighten or whiten the skin as well as to even-tone and brighten the skin; while for Caucasian white skin the focus is on even-toning and brightening the skin. For all skin types, the skin lightening agents can be used to treat pigmentation disorders such as freckles, pregnancy masks and age spots. The purpose of this paper is to compare the various aspects of the four popular skin lightening agents, i.e., kojic acid, kojic dipalmitate, arbutin and magnesium ascorbyl phosphate (MAP), as well as hydroquinone.

SKIN COLOR AND MELANIN:

Skin color is mainly determined by the amount of melanin present in the skin. Melanin is synthesized in melanocytes which are normally found in the epidermal basal layer. Within the melanocytes melanin is bound to a protein matrix to form melanosomes. In the melanosomes, tyrosinase converts tyrosine to eumelanin or pheomelanin. Fig. 1 illustrates the pathways of melanin biosynthesis in the melanocytes. By blocking at the various points of the pathways, skin lightening agents can inhibit or even reverse melanin biosynthesis, and are thus useful in whitening or lightening the human skin. Skin lightening agents can also be used to treat local hyperpigmention or spots which are caused by a local increase in melanin synthesis or uneven distribution.

SKIN LIGHTENING AGENTS:

A. Mechanism of Action: Arbutin, kojic acid, kojic dipalmitate, MAP are all tyrosinase inhibitors. Arbutin reportedly works by competing with DOPA at its receptor site on tyrosinase,[1] while kojic acid inactivates tyrosinase by chelating with its vital copper ion and suppressing the tautomerization from dopachrome to DHICA.[2] L-ascorbic acid and its derivatives, which include MAP, are believed to act as reducing agents on melanin intermediates, thus blocking the oxidation chain reaction at various points from tyrosine/DOPA to melanin.[3] Kojic dipalmitate is a tyrosinase inhibitor, but the exact mechanism of action is unclear. Hydroquinone is also a tyrosinase inhibitor.[4, 5] In addition, hydroquinone has cytotoxic effect on melanocytes, which causes high toxicity to the skin.

B. Ease of Formulation and Stability: Kojic Acid, arbutin and MAP are all water-soluble, and can be easily incorporated into the formulation. On the other hand, kojic dipalmitate is oil-soluble, and can be easily melted into oil phase at 75-85°C, followed with immediate emulsification. However, kojic acid, hydroquinone and arbutin have color stability problems, which impose various difficulties on formulation.

Kojic acid usually turns yellowish brown with time in the finished product. The reasons for this instability are mainly two-fold: a) kojic acid chelates with many metal ions, especially for iron (Fe^{3+}), to produce colored complex (yellow color for iron); b) kojic acid can oxidize slowly in contact with air, and this process accelerates at high temperature. Hydroquinone solution becomes brown on exposure to air as a result of oxidation and must be stabilized. Arbutin has by far higher stability than hydroquinone,[6] but still has potential instability in formulation. MAP is stable in formulation. Kojic dipalmitate is stable to heat, light, pH and oxidation. Compared with kojic acid, kojic dipalmitate neither chelate with metal ions and nor oxidize in air. As a result, kojic dipalmitate has excellent stability in formulation.

C. Safety and Efficacy: Kojic acid is non-toxic and has minimal irritation.[7] Kojic acid has been used in Japan since 1988 and has extended to other parts of the world. The human patch test on kojic dipalmitate showed that it is completely non-irritating.[8] There are no known safety issues associated with the use of kojic dipalmitate. Hydroquinone can be irritating and cause redness and burning. More importantly, it has been shown to cause exogenous ochronosis,[9, 10] which has resulted in a ban on its use in South Africa, Thailand and other countries. Arbutin has far higher safety than hydroquinone, has no irritation and virtually no sensitization.[11] MAP is a vitamin C derivative, has an excellent safety record.

Figure 2 shows the comparison of tyrosinase inhibitory effects among kojic acid, hydroquinone and the blank (water). The y-axis is absorbency at 475 nm which measures the coloration of various melanin

intermediates, and thus tyrosinase activity. The x-axis is the lapse of time starting from the addition of tyrosinase into the culture medium. Figure 2 exhibits that the effect of kojic acid is much milder than hydroquinone while kojic acid has substantial inhibitory effects on tyrosinase activity compared with blank. Figure 3 shows the inhibitory effects of kojic acid and its esters on tyrosinase activity, and reveals that the kojic acid esters have far better inhibitory effects than kojic acid, while kojic acid showed substantial inhibitory effects on tyrosinase activity compared with blank (water). It is clear from Fig. 2 and 3 that kojic esters have comparable or better inhibitory effects on tyrosinase activity than hydroquinone, which in turn has stronger inhibitory effects than kojic acid. In clinical trials on patients with pigmentary disorders, a 55% effective rate was obtained for MAP, [12] while 60-95% and 80% effective rates were obtained for kojic acid[7] and kojic dipalmitate,[13] respectively. It should be noted that both the active skin lightening agent and the formulation itself affect the efficacy or effectiveness of the final product.

D. Functionality and Compatibility: Apart from being a skin lightener, kojic acid is used as an anti-microbial agent to preserve vegetables, kojic dipalmitate is an emollient while MAP is an anti-oxidant. MAP also works synergistically with vitamin E. On the other hand, hydroquinone, kojic acid, arbutin and MAP may not be compatible with some organic sun screens and preservatives due to potential hydrogen bonding, while kojic dipalmitate is fully compatible with all sunscreens and preservatives.

CONCLUSIONS:

The various aspects of the skin lightening agents can be summarized in Table 1 below. It is clear that the use of hydroquinone is no longer desirable, due to its safety concerns. Among the other four skin lightening agents, kojic dipalmitate is the latest development and offers the best overall performance. It is therefore considered the "active of choice" for use in cosmetic formulations for skin lightening purposes.

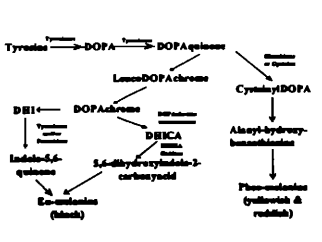


Fig. 1. Melanin biosynthesis pathways.

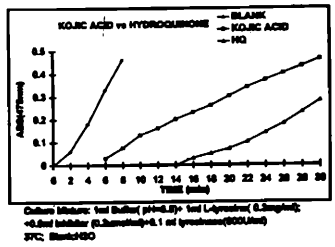


Fig.2. Kojic acid and hydroquinone

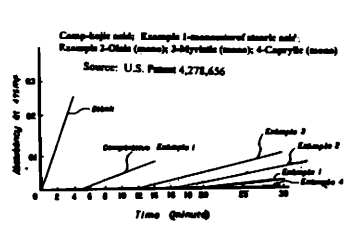


Fig. 3. Kojic acid and its esters

Table 1. Comparison of Skin Lightening Agents

Agent	Hydroquinone	Arbutin	Kojic Acid	Kojic Dipalmitate	MAP
Functionality	Skin Lightening	Skin Lightening	Skin Lightening, Antimicrobial	Skin Lightening, Emollient	Skin Lightening, Anti-Oxidant
Mechanism of Action	Tyrosinase inhibitor	Tyrosinase inhibitor	Tyrosinase inhibitor	Tyrosinase inhibitor	Tyrosinase inhibitor
Ease of Formulation	+	+++	++	+++++	+++++*
Stability	++	+++	+++	+++++	+++++
Irritation	+++	-/+	+	-	-/+
Efficacy	+++++	++++	++++	+++++	++++
Safety	+	++++	++++	+++++	+++++
Cost	+	+++++	++	+++	+++++

* "+++++" indicates the highest value; "-/+" minimal; and "-" negative.

[1] G. Imokawa et al, *J Soc Cosm Chem Jpn* **17** (3), 149 *1993.*
[2] G. Imokawa et al., *J Inv Derm* **91**, 106, 1988.
[3] Y Ohmori et al, *J Soc Cosm Chem Jpn* **18** (4), 215, *1994.*
[4] C. R. Denton et al., *J. Invest. Dermatol.*, **18**, 119, *1952.*
[5] S. Iijima et al., *J. Invest. Dermatol.*, **28**, 1, *1957.*
[6] Japanese Patent, Publication No. 60016906, Publication Date: 850128, "External Drug for Skin".
[7] Y.F. Yang, *China Surfactant Detergent and Cosmetics (Daly Chemical Industry)*, No. 1, p28, 1995
[8] C. F. Liu, X. L. Ye, Unpublished Data, 1995.
[9] H.F. Jordaan, D.G. Van Niekerk, *Am J Dermatopathol* **13**(4), 418-424, 1991.
[10] J. I. Phillips, C. Issacson, H. Carman, *Am J Dermatopathol*, **8**(1), 14-21, 1986.
[11] Japanese Patent, Publication No. 60056912, Publication Date 850402, "External Use Preparation for Skin".
[12] K. Kameyama et al., *J Am Acad Dermatol* **34**(1): 29-33, 1996.
[13] S. Nagai T. Izumi, US Patent 4,278,656, July. 14, 1981.

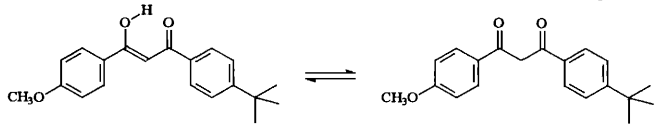
AVOBENZONE PHOTOSTABILITY IN SIMPLE POLAR AND NON-POLAR SOLVENT SYSTEMS

C. Bonda, P. Marinelli, J. Trivedi, S. Hopper, G. Wentworth

The C.P. Hall Company, Chicago, IL 60606

Introduction

Avobenzone (Parsol® 1789, Roche) is the leading organic UV-A filter worldwide. Recent approval by the U.S. FDA likely means usage will increase substantially.[1] Unfortunately, avobenzone's absorbance of UV radiation tends to decrease as its exposure to sunlight increases.[2] Researchers have identified two explanations for this tendency. The first is photolysis wherein UV radiation catalyzes fragmentation of the molecule.[3] The second is UV-induced conversion of this β-diketone from the enol to the keto tautomer (**Figure 1**).[4] In all known solutions, the enol predominates and is the species responsible for absorption in the solar UV range. The keto tautomer absorbs UV below the range found in sunlight.



Enol
λ_{max} = 350-355nm

$C_{20}H_{22}O_3$
mw = 310.39

Keto
λ_{max} = 260-265nm

Fig. 1

Our research measured UV-induced tautomerization in the non-polar solvent cyclohexane when it has been modified by the addition of small amounts of other materials. We were especially interested in finding if polar materials are more stabilizing than protic materials, or vice versa. In this way we sought to increase our understanding of the behavior of avobenzone in sunlight, and perhaps to find ways to mitigate its loss of absorbance in the solar UV range.

Methods

Avobenzone was procured from Roche. Octyldodecanol (Isofol® 20) and octanol (Alfol® 8) were procured from Condea-Vista, and butyloctyl salicylate (HallBrite™ BHB) was provided by C.P. Hall. The solvents and diethyl adipate were procured from Aldrich. The ^{1}H NMR studies were performed on a Bruker AM-400 spectrophotometer. UV radiation (290-400 nm) was provided by a 16S Solar Simulator equipped with a WG 320 filter and PMA 2100 Automatic Dose Controller (Solar Light Co.). The standard radiation dose in these experiments was 35 MED (735 mJ/cm^2). UV spectra were measured on a CECIL CE 3021 spectrophotometer (Buck Scientific). Experiments were applied to 10 ppm avobenzone solutions in the following diluents: neat isopropanol; neat cyclohexane; 99% cyclohexane/1% isopropanol; 99% cyclohexane/ 1% tetrahydrofuran; 99% cyclohexane/ 1% octyldodecanol; 99% cyclohexane/ 1% butyloctyl salicylate; 99% cyclohexane/ 1% diethyl adipate; 99% cyclohexane/1% octanol.

Results

The enol-keto equilibrium in deuterated cyclohexane was determined by NMR to be approximately 97%- 3%. We observed evidence of UV-induced tautomerization from the enol to the keto form in all solutions (**Figures 2, 3**). After irradiation was stopped, we observed in all solutions that absorbance of the keto immediately started to decline and, correspondingly, absorbance of the enol immediately started to rise (**Figure 4**). This process continued until a new equilibrium was reached. There was some, apparently permanent reduction in total avobenzone concentration. As summarized in **Table 1**, hydroxylic (and, therefore, protic) additives such as primary alcohols and butyloctyl salicylate were more successful at inhibiting enol-keto tautomerization than were aprotic polar additives and non-polar additives which were also aprotic.

Rank	Diluent	% Original Absorbance after 35 MED (@ 353nm)	Molecular Weight of diluent additive
1	99% cyclohexane/1% IPA	91.2%	68
2	Neat isopropanol	90.9%	—
3	99% cyclohexane/1% octanol	88.7%	130
4	99% cyclohexane/1% butyloctyl salicylate	84.5%	306
5	99% cyclohexane/1% octyldodecanol	78.9%	298
6	99% cyclohexane/1% tetrahydrofuran	69.2%	72
7	Neat cyclohexane	61.8%	—
8	99% cyclohexane/1% diethyl adipate	58.8%	174

Table 1

Conclusions

The tendency of avobenzone to lose absorbance in sunlight is explained in part by UV-induced enol to keto tautomerization. Surprisingly, the tautomerization reverses when irradiation ceases, and something resembling the original enol-keto equilibrium results. Stability during UV irradiation of the enol-keto equilibrium is increased in what appears to be a concentration-dependent manner by adding hydroxylic (protic) materials to the solvent. Butyloctyl salicylate increases stability of the enol-keto equilibrium disproportionately when compared to the nearly equimolar octyldodecanol. Polar materials which are not hydroxylic do not appreciably improve stability of the equilibrium.

Our hope is that these findings will lead to improvements in avobenzone formulations and, therefore, to more protective, dependable, and efficacious sunscreens.

References

1. Food and Drug Administration, Announcement of Enforcement Policy, *Fed. Register,* Vol **62**, No. 83, April 30, 1997
2. C. Bonda, Formulating Stable, High SPF, Broad Spectrum Sunscreens with Avobenzone, from poster presented at SCC Annual Scientific Meeting, NYC, (*1997*)
3. W. Schwack and T. Rudolph, Photochemistry of Dibenzoyl Methane UVA Filters Part I, *J. Photochem. & Photobiol.*, **28**, 229-234, (*1995*)
4. L. Andrae et al, A UVA Filter (4-*tert*-butyl-4'-methoxydibenzoylmethane): Photoprotection Reflects Photophysical Properties, *J. Photochem. & Photobiol.*, **37**, 147-150, (*1997*)

Acknowledgements

The authors gratefully acknowledge the valuable assistance and contibutions of Yin Hessefort, Magda Roth, Urvil Shah, and Robert McMillin of the C.P. Hall Company, Dr. John Harwood of the University of Illinois-Chicago, and David Steinberg of Steinberg & Associates.

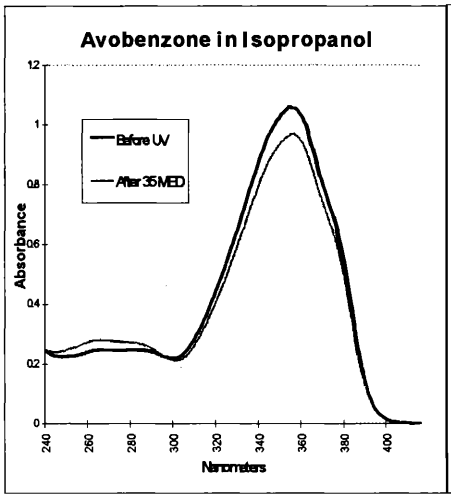


Fig. 2

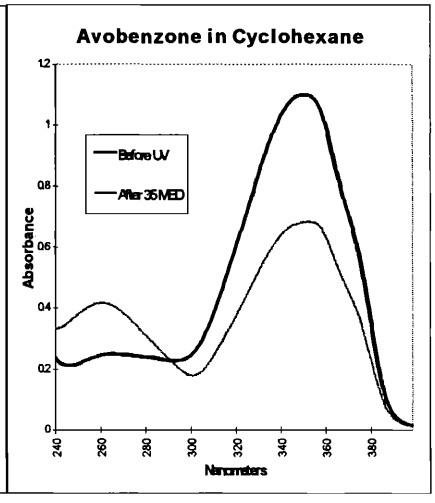


Fig. 3