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SELECTING THE OPTIMUM SILICONE PARTICLE SIZE/CATIONIC POLYMER STRUCTURE TO MAXIMIZE SHAMPOO CONDITIONING PERFORMANCE

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Summary:

Cationic hydroxyethylcellulose (HEC) is widely used to deposit and improve the conditioning performance of silicone emulsions in shampoos. In this study, two silicone emulsions of similar viscosity but different particle size, 0.5μm and 20μm, were tested with a variety of cationic HEC polymers. For the silicone with the smaller particle size, the high molecular weight cationic HEC polymer deposited more silicone onto both damaged and undamaged hair than the lower molecular weight polymer. For the silicone emulsion with the larger particle size, the cationic HEC with the lower molecular weight deposited more silicone than the cationic HEC with high molecular weight. Although the large silicone droplet can be easily deposited onto undamaged hair and damaged hair without a deposition aid, the deposition is uncontrolled and variable. Cationic HEC helped to control silicone deposition, yielding consistent, predictable deposition and maintaining good overall conditioning performance. Panel studies showed that the amount of silicone deposited correlated well with conditioning performance. Scanning Electronic Microscope (SEM) analysis confirmed the deposition differences on hair surface.

Background:

Silicone polymers, especially non-ionic silicone polymers have been commonly used in personal care products for over 50 years (1). Their unique, low surface tension allows easy spreading on keratinous surfaces, such as hair and skin, and provides significant beneficial improvements in sensory properties, such as soft feel and shine. Silicone emulsions are predominantly used because they are easier to handle and formulate than silicone oil. Silicone prepared with the proper emulsifier results in a stable emulsion having a defined particle size. The delivery of silicone to substrates directly from emulsions or from surfactant-containing 'rinse off' formulations has been studied. The deposition of the silicone onto hair has been reported to depend on the ionic charge, droplet size and viscosity of the silicone oil emulsion (2, 3).

Polycations, such as cationic HEC, are widely used either as conditioning polymers by themselves or in conjunction with other oleaginous materials in both hair and skin care products. The deposition of cationic HEC and silicone from shampoo systems onto hair has been reported (4, 5). The cationic HEC forms a coacervate (polymer-surfactant complex) upon dilution during the application and rinsing of the formulation (6). The coacervate phase separates from the bulk formulation and is deposited on the hair or skin. Insolubles, such as silicone, are entrapped in the coacervate phase and become deposited along with the polymer. The properties of the coacervate are dependent on the surfactant system and polymer affecting the combined deposition of insoluble actives and cationic polymers onto keratinous substrates (6, 7). There is no detailed study of the effect of the silicone particle size, in conjunction with polymer parameters, surfactant system, and hair type on deposition of silicone onto hair. In this study, two nonionic silicone emulsions of high molecular weight polydimethylsiloxane with different particle sizes, 0.5μm and 20μm, were evaluated with a variety of cationic HEC polymers, hair types and two surfactant systems: (A) 15.5% sodium laureth sulfate (ES-2) / 2.6% disodium cocamphodiacetate (DSCADA).; and (B) 4% ammonium lauryl sulfate (ALS) / 13.5% ammonium laureth sulfate (ALES) / 2.6% cocamdiopropylbetaine (CAPB) / 1% sodium chloride. The results indicate that choosing appropriate polymers in conjunction with silicone particle size is critical for formulating "2-in-1" conditioning shampoo having optimum conditioning performance. More important, a simple change in polymer or silicone particle size allows formulators the flexibility to "dial in" desired sensory properties.

Experimental Results:

Table I: Formulations for study:

Surfactant	*A: ES-2/DSCADA*[3]					*B: ALS/EA-3/CAPB/NaCl*[3]			
Particle size[4] *Polymers*	*No polymer*	*LH*[1]	*HL*	*HH*	*PQ-67*[2]	*No polymer*	*LH*	*HL*	*PQ-67*
0.5u	*E-I*	*E-III*	*E-V*	*E-VII*	*E-IX*	*A-I*	*A-III*	*A-V*	*A-VII*
20u	*E-II*	*E-IV*	*E-VI*	*E-VIII*	*E-X*	*A-II*	*A-IV*	*A-VI*	*A-VIII*

(1) For cationic HEC polymers, the first abbreviation indicates molecular weight and the second abbreviation indicates cationic charge. "H" means high and "L" means low.

(2) PQ-67 is hydrophobically modified cationic HEC of high molecular weight with low cationic charge.

(3) All formulations contain 0.25% polymer, except control, and 1% silicone emulsion.

(4) Small particle size silicone emulsion and large particle size silicone emulsion are DOW Corning® 2-1352 Emulsion and DOW Corning® 2-1491 Emulsion, respectively.

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Silicone deposition on hair - The total amount of silicone deposited on hair was measured on hair that was treated five times with the prototype formulation listed in Table I. The hair was rinsed and dried between each treatment. Two types of undamaged hair, European brown hair and Asian hair, and one type of damaged hair, commercial bleached hair (all from International Hair Importers and Products Inc.), were used for this study. The silicone was extracted from the hair by a 50/50 (v/v) methyl butyl ketone / toluene solution, and then measured using atomic absorption spectrophotometry. The results using both surfactant "A" and "B" showed that the structure of the cationic HEC - especially the molecular weight - the silicone droplet size and type of hair all had a very strong impact on silicone deposition. In Figure 1, the formulation containing small silicone particle size (0.5μm) without cationic HEC, such as Formula E-I gave very poor silicone deposition on all types of hair. However, the formulation containing cationic HEC improved the silicone deposition. For example, the cationic HEC with high molecular weight (HL in Formula E-V), delivered more silicone than the low molecular weight polymer (LH in Formula E-III). The hydrophobically modified cationic HEC, PQ-67, deposited even more silicone on the hair, especially damaged, commercial bleached hair. This trend was reversed with the formulations containing large particle size silicone.

Figure 1: Deposition of Small Silicone Particle from Surfactant A in Different Types of Hair

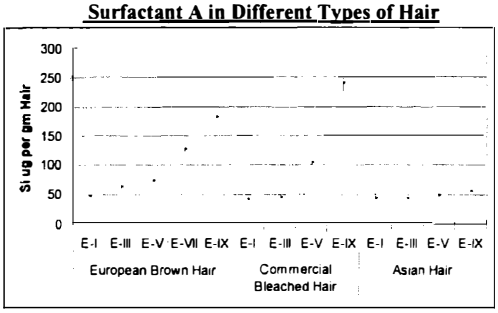
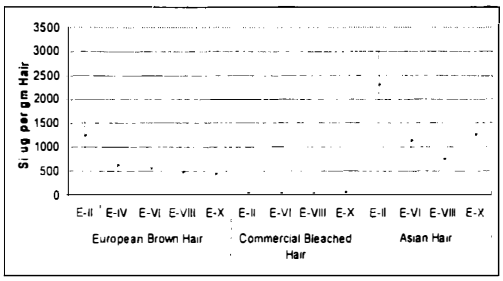


Figure 2: Deposition of Large Silicone Particles from Surfactant A on Different Types of Hair



In Figures 2 and 3 the formulations containing large silicone particle size (20μm) without cationic HEC (Formulas E-II and A-II) yield very high silicone deposition on undamaged hair, such as European brown hair and Asian hair. This phenomenon can be explained by the fact that the attractive Van der Waals forces between particles increase with particle size and, eventually, the large particles tend to adhere better when they collide with another surface (6). However, the silicone deposition measurement shows a big variation of silicone deposition on different hair tresses. This indicates the deposition is uncontrollable and could lead to inconsistent conditioning and eventual silicone build-up. Conversely, the formulation containing cationic HEC yields more consistent and controlled deposition. The data also suggests that when large particle size silicone is used, low molecular weight cationic HEC should be used to maximize deposition onto undamaged hair. This difference in deposition profile may be due to high molecular weight polymers stabilizing the large silicone droplets and preventing them from colliding with the hair surface.

The large particle size silicone, however, is relatively easier to deposit onto undamaged, European brown, hair than damaged, commercial bleached, hair regardless of which polymer is used. The bleaching process has significantly modified the hair surface of the commercial bleached hair to a highly negatively charged surface which potentially affects the silicone deposition process.

The silicone deposition from the formulation of Surfactant B containing large silicone particles shows a similar trend as the formulation from Surfactant "A" (Figure 2 & 3). However, the relative amount of silicone deposited onto European brown hair from surfactant "B" is much lower than surfactant "A". This demonstrates that the surfactant system can significantly change the silicone deposition process.

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Figure 3: Silicone deposition on European Brown Hair (Large Silicone Particle Size) from Surfactant B

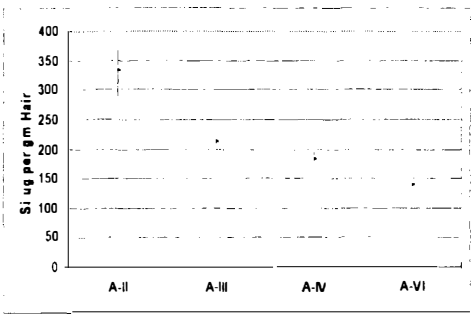
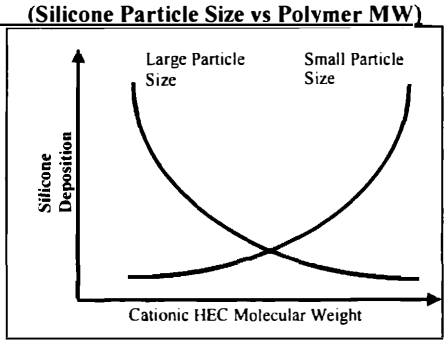


Figure 4: Summary of Silicone Deposition (Silicone Particle Size vs Polymer MW)



The above data suggests that the silicone deposition from silicone containing shampoos onto the hair is a very complex process. Many factors, such as silicone droplet size, cationic polymers, surfactant system and hair type can change the deposition process. The general trend of silicone deposition with different silicone particle sizes and different cationic HEC polymer molecular weight is summarized in Figure 4. In general, more large particle size silicone is deposited by low molecular weight HEC, while more small particle size silicone is deposited by high molecular weight HEC. One of the major roles of cationic HEC in a conditioning shampoo is to control the silicone deposition in order to maintain good overall conditioning performance. Thus, formulators need to select the right combination of silicone particle size with the structure of cationic HEC in order to deliver the desired amount of silicone onto the hair.

Conclusion:

This study demonstrates the importance matching the optimum silicone particle size and cationic polymers structure in order to deliver the desired amount of silicone onto a specific type of hair in a predictable way to maximize the conditioning shampoo performance without causing a silicone buildup issue.

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MALASSEZIA AND SEBORRHEIC DERMATITIS: ETIOLOGY AND TREATMENT

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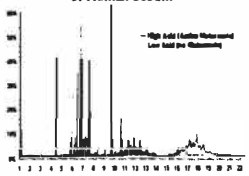
Etiologic Mechanism of Dandruff and Seborrheic Dermatitis (D/SD)

Dandruff and seborrheic dermatitis are chronic clinical scalp conditions affecting greater than 50% of the population, the primary symptom of which is visibly excessive scalp scaling. Seborrheic dermatitis is a more severe disorder which includes increased desquamation of areas other than the scalp and visible inflammation[1]. D/SD are more than just superficial disorders of the stratum corneum, including alteration of the epidermis with hyperproliferation, excess intercellular and intracellular lipids, interdigitation of the corneal envelope, and parakeratosis[2,3]. Combination of several recent lines of investigation points out a novel mechanism for the etiology of D/SD. Scalp *Malassezia* degrade sebum, freeing multiple fatty acids from triglycerides. They consume the specific saturated fatty acids necessary for their proliferation, leaving behind the unsaturated fatty acids. The free fatty acids then penetrate the scalp skin, and in susceptible individuals breach the skins barrier function and induce a hyperproliferative response[4].

The role of Sebum

Malassezia Mediated Degradation of Human Sebum



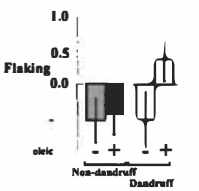
Human sebaceous glands (SG) are found over the entire skin surface (except the palms of the hands and soles of the feet), but sebum secretion is highest on the scalp, face, chest, and back[5]. Sebum is produced under hormonal control, with SG active at birth under the control of maternal androgens. They quickly reduce in size and sebum production until puberty. As puberty begins the SG activate, this time under the control of circulating androgens. The sebum secretion rate increases throughout the teens, remains steady through the twenties and thirties, then lessens with age[1,6]. D/SD show a strong temporal correlation with SG activity, following the pattern of early cradle cap, low incidence until puberty, increasing incidence through the teens, 2nd and 3rd decades, then declining[1,4,7]. Human sebum is a complex mixture of triglycerides, fatty acids, wax esters, sterol esters, cholesterol, cholesterol esters, and squalene. As the sebum is secreted, it consists primarily of triglycerides and esters, which are broken down by commensal microbes into diglycerides, monoglycerides, and free fatty acids. Human sebum contains both saturated and unsaturated fatty acids, with a preponderance of unsaturates. The fatty acid chain lengths of human sebum vary considerably, but are predominantly 16 and 18 carbons. The role of specific fatty acids of human sebum becomes apparent when we examine the metabolism of *Malassezia*.

Role of *Malassezia*

Over one hundred years ago, Malassez implicated the yeast *Malassezia* in the etiology of dandruff[8]. While *Malassezia* are not numerically correlated to dandruff and seborrheic dermatitis (D/SD), recent evidence strongly supports their causal role[4]. This evidence includes the effectiveness of multiple chemical entities whose sole common mechanism of action is antifungal activity, as well as the very distinct numerical correlation of reduction in severity with reduction of *Malassezia* numbers[9], and there is now general agreement as to their causal role[4,10]. Interestingly, *Malassezia* have a very specific taste for individual fatty acids[11,12]. The *Malassezia* lipases are non-specific and degrade any available triglycerides (Fig. 2). The saturated fatty acids are consumed, and the abundant unsaturates, predominantly oleic (C18:1Δ9) and palmitoleic (C16:1Δ9) are left on the skin (Fig. 2, Fig.3). Recently, novel molecular methods have overcome the difficulties presented by culture of *Malassezia*, and the specific *Malassezia* species present on human scalp have been elucidated[4,13], and the most common species on human scalp are *M. restricta* and *M. globosa*.

The Role of Individual Susceptibility

Role of Individual Sensitivity



It has now been shown that a representative *Malassezia* fatty acid metabolite (oleic acid) is able to induce scalp flaking in susceptible individuals, but not in non-susceptible individuals (Fig.2)[14]. The *Malassezia* most commonly found on human scalp, *M. globosa* and *M. restricta*, non-specifically degrade human sebum and release unsaturated fatty acids like oleic and palmitoleic onto the scalp (Fig. 1). This strongly supports that dandruff sufferers display an underlying defect in permeability barrier function that renders them more susceptible to fatty acid-induced barrier disruption. In this regard, dandruff-susceptibility may be determined, at least in part, by a defect in

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basal permeability barrier function as is well established in the case of atopic dermatitis. This also explains the lack of a simple quantitative relationship between *Malassezia* species and D/SD presence or severity. Integrating all available data, it appears that dandruff and seborrheic dermatitis most likely results from three specific etiologic factors: 1 - Individual susceptibility; 2 - Sebaceous secretion and; 3 - *Malassezia* fungi. Individual susceptibility is most likely related to basal permeability barrier function, immune system function, and possibly even the overall microbial community.

Treatment

As D/SD are the result of sebum secretion, fungal activity, and innate susceptibility, the easiest, safest, and most effective treatments remain anti-fungal shampoos. The most common anti-fungal materials in use today are pyrithione zinc (ZPT), selenium sulfide (SexSy), ketoconazole, and to a lesser extent climbazole and octopirox. As rinse off products leave only small amounts of active material, in the microgram per square centimeter range, much caution must be taken with pharmacology. The deposition profile must be adequate for the materials potency, the residual material must be deposited in the appropriate location in a bioavailable manner, and the product must be cosmetically appealing enough for the patient to continue use. Several ZPT particles with differing deposition and bioavailability profiles will be discussed.

Conclusions

The common etiology of D/SD is therefore a convergence of three factors: 1) SG secretions, which provide the substrate for *Malassezia* growth; 2) *Malassezia* metabolism of the sebaceous secretions, releasing irritating unsaturated fatty acids; and 3) individual susceptibility to the penetration of the fatty acids and the resultant inflammation. The optimal and most efficacious method for treatment is anti-fungal actives delivered from rinse-off products. The low deposition inherent in rinse-off products places significant constraints on the potency of the active material and the delivery pharmacology of the product chassis.

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HYDRATION EFFECT ON HUMAN NAIL PERMEABILITY

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Statement of purpose: Onychomycosis is the #1 nail disorder diagnosed and treated by podiatrists and it accounts for up to 50% of nail diseases. Up to 13% of population of the United States is estimated to have dermatophyte onychomycosis. Over the years treatment of onychomycosis has changed from nail avulsion to pharmacotherapy with oral anti-fungals and nail lacquer. A desirable characteristic of a topical antifungal agent in the treatment of onychomycosis is the ability of the drug to penetrate the keratin of the nail. However, topical treatment alone is generally unable to cure onychomycosis because of insufficient nail plate penetration. The effects of water on keratinized tissues, including horn, wool, hair and stratum corneum are studied to some extent, showing that the mechanical and transport properties are related to water content. While this information is fundamental, literature review shows a paucity of data with respect to human nails. The magnitude of the effect observed in other tissues suggests that hydration may be an important determinate of ungual drug delivery, e.g., for topical treatment of nail dystrophies caused by onychomycosis, nail psoriasis and paronychia. The understanding and knowledge of hydration effects on nail permeability may be useful clinically and cosmetically in topical treatment of nail disorders. The purpose of this study is to clearly define the effects of hydration on human nail permeability to water, i.e., to quantify transport parameters for water in human nail over a complete range of hydration.

Method: We performed vapor phase water sorption-desorption studies at 32°C and 7 different water activities ranging from 15-100% using cadaver finger nails and tritiated water (^3H_2O) as a probe. Environmental relative humidity was controlled with various concentrations of H_2SO_4, NaCl, K_2CO_3, and LiCl solutions. [1] Human nail samples (3 donors with n=2/donor) free of any adherent tissue were cleaned with a mild detergent solution and dried at 45°C. Dry human nail samples were allowed to sorb over (in vapor phase) tritiated water of radioactive concentration 10 μCi/ml. The nail tissue was weighed periodically to determine whether equilibrium was achieved. At the end of the sorption phase the nail samples were moved periodically over solutions (in vapor phase) with no radioactive compound and the rate of desorption of radioactivity from the nail was measured. Desorption was studied until no radioactivity was detected in the receptor solutions. The vapor phase assembly was replaced between the sorption to desorption phase to eliminate errors resulting from radioactive compound being trapped in the assembly. Radioactivity was measured using liquid scintillation counting.

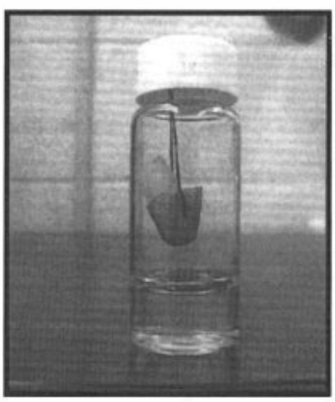

Figure 1: Schematic diagram of the vapor phase sorption-desorption study

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Results and conclusions: The rate and extent of ^3H_2O desorption by the nail samples at each RH was calculated in terms of adsorption volume, *v*, expressed as (g of water) / (g of dry tissue). Figure 2 shows that the amount of tritiated water desorbed from nail plates decreased with the decrease in water activity. Preliminary analysis of these data using a mathematical solution for desorption of a solute which is uniformly distributed in a homogenous membrane, into a stirred solution with sink conditions, [2] yields diffusivity values from 7.3 X 10^{-10} cm^2/s (for $a_w = 0.15$) to 3.6 X 10^{-8} cm^2/s (for $a_w = 1$); thus hydration increased diffusivity of water in nail. The desorption rate obtained is a combination of diffusion in the nail tissue and diffusion in vapor phase. A more detailed analysis accounting for the time lag in the vapor phase and tritium exchange with nail proteins is underway.

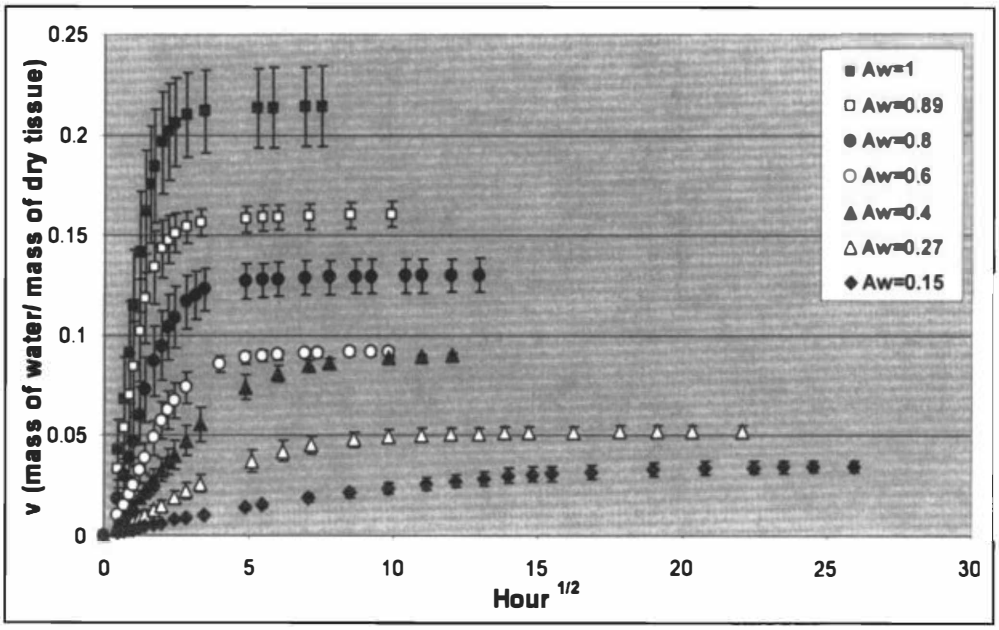

Figure 2: Desorption of ^3H_2O from human cadaver nail plates

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NANO-SIZED HYBRID CAPSULES BASED ON CYCLODEXTRIN ESTERS: A TOTALLY NEW FLUIDIC ORGANIZATION FOR PENETRATION ENHANCEMENT OF COSMETIC ACTIVE COMPOUNDS

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**Janice Hart is presenting on behalf of Engelhard-Lyon*

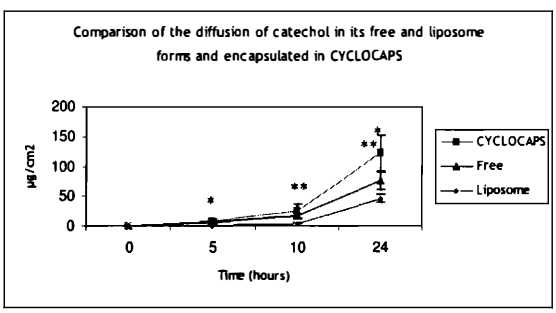
Nanotechnologies promised for human being future, a better life as far as health management and medical applications are concerned. Cosmetic companies have been, in this area, one step ahead and have already developed fantastic applications using nanocapsules or nanospheres that are not yet used in the pharmaceutical area.

The aim of our study was to develop a chemically modified cyclodextrin that would be able to associate spontaneously into nano-size range biovector, able to mimic the fluidic membrane of liposomes, and able to stimulate the penetration of encapsulated active compounds. The concept of this development was linked with the idea that such type of biovector would induce a double encapsulation system, the encapsulated active compounds being both in the cavity of the cyclodextrin and inside the nanoparticle formed with those nanostructures as well.
The fluidic membrane of this biovector enhances the penetration ability.

Methods and Results:

Progressive chemical esterification was employed using 3 to 15 equivalent of lauric fatty chains for one equivalent of beta-cyclodextrin. Nanoparticles were produced using the solvent evaporation technique. They were analysed for their size (N4 Plus, Beckman Coulter), their shape (using Atomic Force Microscopy, Explorer Thermomicroscope) and for their encapsulated active compounds using conventional HPLC techniques.

Penetration of encapsulated active compounds (catechol, 0.1%, p/p) between those structures (called CYCLOCAPS®, Engelhard, Lyon, France), was then compared with a solution of free catechol and standard liposomes, using Franz cell diffusion devices and rat skin biopsies.
Diffusion of each product was estimated at 5, 10 and 24 hours by measuring fluorescence (276/298) in the medium in the lower compartment of the Franz cell constituted by PBS with ethanol 80/20.



Cyclocaps 24H:
* statistically different ($p<0.05$) from free form
** statistically different ($p<0.01$) from liposome form
Cyclocaps 10H:
** statistically different ($p<0.01$) from liposome form
Cyclocaps 5H:
*statistically different ($p<0.05$) from liposome form

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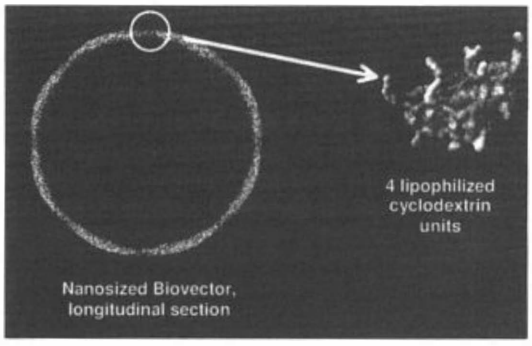
Trans-cutaneous penetration studies have shown that this new biovector induces a very good penetration of active compounds inside the deep part of the skin, which is a property particularly interesting for substances developed for instance for their ability to promote extracellular matrix synthesis, or to inhibit MMPs or tyrosinase activities. Moreover, for the active compounds that have been tested during these studies, penetrations were far above the ones obtained with standard liposomes, which makes those fluidic nanoparticles very interesting for their cosmetic activities.

Observations of those nanoparticules were also performed into suspension using fluorescent microscopy (Axioskop2+, Zeiss), with a rhodamine filter (546/590), after encapsulation of Octadecyl Rhodamine (Molecular probes, USA).

The particle size last from 100 to 300 nm according to the nature of the active compound that is encapsulated.

The stability of size and shape has been controlled into suspension for more than 2 years.

Mathematical modelisation was performed using advanced modelisation computer models using Turbo-Frodo software and docking experiments to evaluate the complex hybrid structure organisation of such capsule membranes.



Encapsulation of different active compounds performed: vitamins (B6, B3, H, B12, B2, A and E), slimming products such as caffeine, theophyllin, carnitine, and Centella Asiatica extract: antioxidants such as ubiquinones; and whitening products such as catechol or lipoïc acid.

Conclusion and perspectives

These new fluidic and fractal nanosized vectors allow an improved performance of encapsulated active compounds devoted for the deeper part of the skin, without providing any side effect. It is a safe biovector, and allows some real breakthrough in the Nanotechnology field that induces a complete modification in the usual classification of nano-biovectors.

The next evolution of those biovectors will be around cell targeting, which could be made by grafting some chemical structures with cell membrane affinities, onto the structure of those nanoparticles

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SIRT1, THE HUMAN HOMOLOGUE TO SIR2, IS EXPRESSED IN HUMAN SKIN AND IN CULTURED KERATINOCYTES FIBROBLASTS AND HaCaT CELLS; AND ITS LEVEL IS CLOSELY RELATED TO STRESS AND AGING

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Introduction

The SIR2 (silent information regulator 2) gene family was first studied in yeast, and it is a family of protein deacetylases (Sirtuins) that are NAD(+)-dependent enzymes. Evidence progressively showed that SIR2, a NAD-dependent histone deacetylase, is the founding member of the family of sirtuins.

Studies in yeast have shown that the SIR2 family has diverse biological functions including gene silencing, DNA repair, cell cycle progression, development, metabolism, apoptosis, heterochromatin formation, and aging.[1-6]

The discovery that SIR2 requires NAD for its activity immediately suggested a link between SIR2 activity and caloric restriction. This link was strengthened by the observation that life span extension by caloric restriction requires the SIR2 protein.[7-10]

The discovery that overexpression of SIR2 is sufficient to extend life span in yeast elevated SIR2 to the central position of aging research in this organism.

In mammalian cells, studies have identified SIRT1 as the homologue of the Saccharomyces cerevisiae chromatin silencing factor SIR2. In the studies that followed, 4 types of human SIRT were identified.

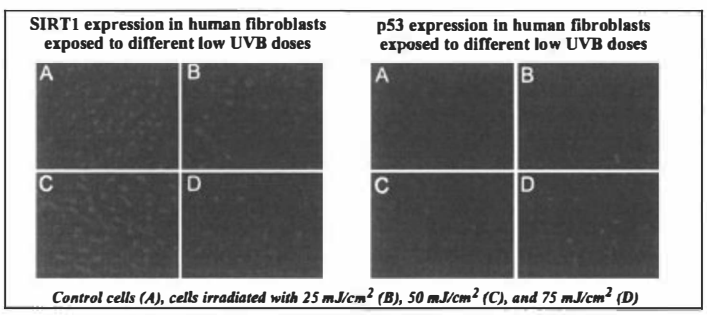
SIRT1 has been found to associate with the tumor suppressor protein p53, and the deacetylation of p53 by SIRT1 has been shown to negatively regulate p53-mediated transcription. Therefore, the role of SIRT1 in preventing cellular senescence and apoptosis induced by DNA damage and stress has been strongly suggested.

Hence, it is becoming increasingly apparent that SIRT1 is a key regulator of cell defense and cell survival in response to stress.

Results

Immunostaining studies of cultured human keratinocytes, HaCaT cells and fibroblasts demonstrated that SIRT1 exhibits a clear nuclear staining in these cells.

Immunofluorescence studies showed that, with low doses of UVB stress, SIRT1 expression increases in a dose-dependent manner, while p53 expression shows very little variation. In contrast, at higher UV doses (above 40-50mJ/cm^2) SIRT1 expression decreases while p53 expression increases significantly.



Control cells (A), cells irradiated with 25 mJ/cm^2 (B), 50 mJ/cm^2 (C), and 75 mJ/cm^2 (D)

This result corroborates SIRT's role in the protection of cells by suppressing p53 after a moderate injury of cells by UVB; after stronger, damaging UV doses, p53 takes over, leading the cell to cell cycle arrest or apoptosis.

mRNA studies confirmed this finding and showed that SIRT1 mRNA increased 3 h after low doses of UVB and lasted for 24 h.

In response to caloric restriction, SIRT1 expression increased, in a dose-dependent manner, to reach maximum expression with total glucose deprivation (for 24 and 48 h), while p53 levels varied very little under these conditions.

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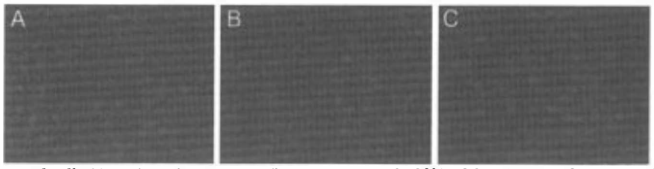
SIRT1 expression in human fibroblasts exposed to caloric restriction



Control cells (A), cells cultured in media containing only 50% of the glucose of the control, for 24 h (B), cells cultured in media without glucose, for 24 h (C)

Studies of SIRT expression in different human and animal tissues (but not the skin) have shown that SIRT1 expression is nuclear.

Our studies on *ex vivo* skin involved comparative studies between frozen and fresh skin samples, and between skin samples at different ages.

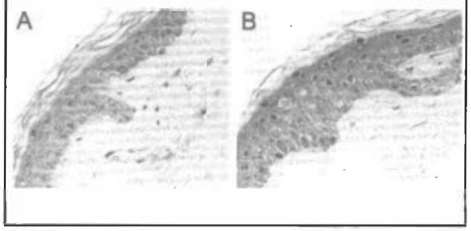
These studies showed that in fresh *ex vivo* skin samples, SIRT1 exhibited a predominant nuclear staining throughout the epidermis. Some cytoplasmic staining was also seen.

SIRT1 expression in fresh human *ex vivo* skin

Comparative studies of skin samples from donors of different ages (30 to 55) did not reveal a significant age-related difference in SIRT1 expression under a stress-free condition.

Interestingly, when skin samples were irradiated with UVB (50-200 mJ/cm^2), fresh skin samples exhibited a clear dose-dependent increase in SIRT1 nuclear expression, up to 100 mJ/cm^2, which correlated with low tissue damage and low p53 expression, whereas high UVB doses yielded strong p53 expression. Amazingly, these results appeared more evident in young skin.

SIRT1 expression in skin from a 30-year-old donor irradiated with UVB



No UVB (A) 100 mJ/cm^2 (B)

Conclusion

These studies demonstrate that SIRT1 is expressed in human skin and in cultured human keratinoctes, and fibroblasts.

These studies also confirm the close relationship of SIRT1 with p53, stress, and cell aging; they show that SIRT1 expression correlates with its ability to protect human skin and is related to UV stress dosage.

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ABSORPTION AND EVAPORATION OF VOLATILE AND POTENTIALLY HAZARDOUS CHEMICALS FROM HUMAN SKIN

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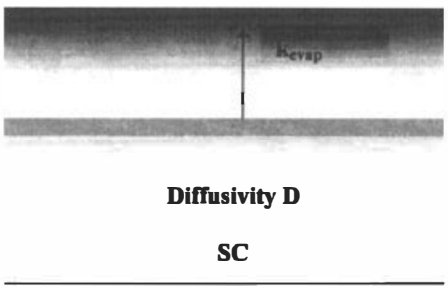
Objective: To test an existing kinetic model for disposition of volatile compounds applied to human skin and to develop a predictive mathematical model based on diffusion theory that improves significantly on current predictions and extends the range of validity to pesticides and non-corrosive industrial solvents.

Introduction: Estimation of penetration rates and systemic absorption of compounds following accidental or intentional application to the skin is an important aspect of risk assessment for cosmetic and personal care products, occupational exposures, environmental exposures, and chemical warfare agents. Previous work in our laboratory has shown that disposition of DEET and benzyl alcohol from human skin ex vivo is adequately described by first-order one and two compartment models. A simple diffusion model with a headspace compartment has also been developed.[1,2] Presented below is an analysis of absorption and evaporation rates of the mosquito repellent DEET based on this model.

Theory: A one-dimensional diffusion model is depicted in Figure 1.[1,2]

Figure 1: Diffusion model for absorption and evaporation of DEET from human skin



V_h = headspace volume,
M_{sat} = saturation dose (C_{sat}•h/10)
k_{evap} = Evaporation mass transfer coefficient
h^2/D = diffusive time constant in SC,
C = concentration in the SC,
SC = stratum corneum

Methods: Split thickness human cadaver skin was mounted on modified Franz diffusion cells connected to a customized volatiles trap using Tenax® cartridges. Diffusion cells, filled with phosphate buffered saline (pH 7.4 with 0.02% sodium azide) were maintained at 37 ± 2 °C. Tissue integrity was ascertained using tritiated water. A 1%w/w solution of ^{14}C-N, N-diethyl-3-methylbenzamide (^{14}C-DEET) was applied to the skin. An air pump attached to the system allowed air to be passed over the skin at a predetermined rate. Vapor was collected by means of a Tenax® absorbent cartridge attached to the evaporation chamber. Samples were collected at 0.25, 0.75, 2, 4, 8, 12 and 24 hours post-dose. Evaporation and absorption of ^{14}C-DEET were measured at varying airflows (v =10-100 mL/min, n = 2-6 per airflow). Receptor solutions were analyzed by liquid scintillation counting (LSC). Tenax® cartridges were thermally desorbed and analyzed similarly. At the end of 24 hours, the tissue was dissolved in Soluene-350R® and analyzed by LSC.

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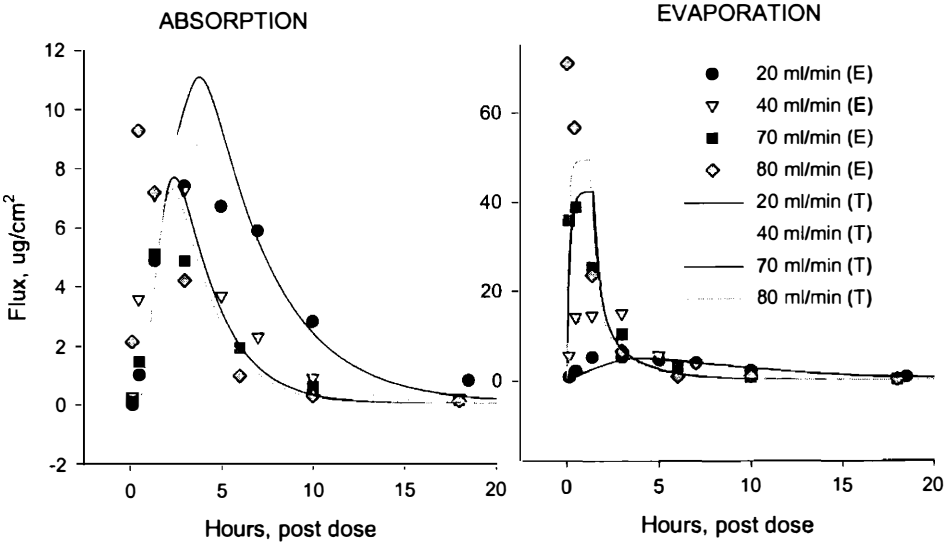
Results: The experimental data are shown in Table 1. Evaporation rate increased in direct proportion to airflow. The percent of dose evaporated after 24 h ranged from 16 ± 4 % at v =10 mL/min to 53 ± 7% at v = 80 mL/min. Absorption and evaporation rates of DEET at selected airflows are depicted in Figure 2.

Table 1: Mass balance of 1%w/w solution of ^{14}C-DEET from human skin in vitro as a percent of dose applied

Airflow mL/min	% Dose evaporated		% Dose absorbed		Para film	Total Recovery
	Cartridge	[a] Et-OH Rinsing	[b] Tissue	Receptor Fluid		
10	15.8±3.8	1.2±0.3	8.0±2.9	68.8±6.6	0.7±0.5	94.5±1.1
20	29.1±5.5	1.4±0.3	7.4±0.6	51.8±6.0	0.0±0.0	89.7±5.1
30	41.3±10.5	1.9±0.4	11.9±4.0	36.7±9.7	0.4±0.2	92.2±4.0
40	42.4±3.6	0.6±0.1	5.9±1.0	32.0±1.4	0.0±0.0	81.0±3.9
50	51.1±14.8	1.4±0.3	13.5±8.3	24.7±6.1	0.0±0.0	90.8±7.0
60	44.9±3.0	0.9±0.2	5.8±1.1	26.5±3.1	0.0±0.0	78.1±1.2
70	58.8±6.7	0.8±0.3	11.3±8.2	20.0±2.6	0.4±0.3	92.0±1.0
80	52.4±7.3	0.7±0.1	13.4±6.1	19.7±1.3	0.8±0.5	87.0±3.6
100	37.6±10.6	0.5±0.2	13.6±9.2	21.6±6.6	1.9±1.8	89.2±3.7

[a] = Ethanol rinsing of evaporation trap, modified Franz® cell top and connecting tubes (where applicable),
[b] = % dose recovered from skin and Para film (where applicable) at end of experiment, i.e, 24/25 hrs.

Figure 2: ^{14}C-DEET evaporation and absorption rates from human skin in vitro as a function of airflow over the diffusion cell. (E) = Experimental values, (T) = Theoretical Prediction



Conclusion: Evaporation and absorption of solvent deposited volatile compounds from skin can be satisfactorily described by a diffusion model employing accessible physical properties and a simple representation of the skin barrier.

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FORMULATING FOR FAST EFFICACY

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Introduction:

The underlying issue in not delivering the promise of cosmetic products in a consumer-noticeable and fast way is very often the lack of sufficient skin delivery of active ingredients into the skin. Our previous work has indicated that the choice of the emollients determines the quantity of active ingredient penetrating the skin. The polarity of the phase in which the active ingredient is solubilized can be calculated and is a compromise between the solubility of the active ingredient in the formulation on the one hand and the driving force for penetration into the skin on the other hand. For details of this so-called *Formulating for Efficacy* concept, see Figure 1 and ref. 1.

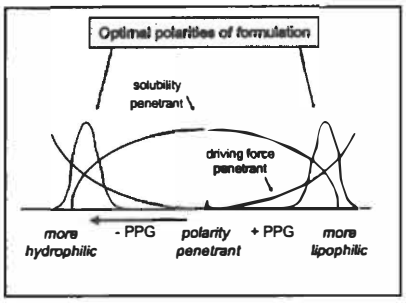

Figure 1: Schematic representation of the "Formulating for Efficacy" Concept

Whereas this approach helps to increase the quantity of active ingredient penetrating the skin, it does not guarantee that at any given time, there is also sufficient active ingredient at the site of action to achieve levels above the minimal effective concentration. To achieve this, the deposition of active ingredient in skin – the so-called drug targeting – should also be taken into account.

Deposition of ingredients is influenced by both the rate and extent of the percutaneous absorption process. Whereas the choice of emollients regulates the extent to which active ingredients penetrate the skin, the choice of the emulsifier influences the rate of skin penetration, probably due to the interaction of emulsifiers with skin lipids. In order to investigate the influence of emulsifiers on the rate and extent of skin penetration of cosmetically active ingredients, two types of studies were performed:

1. Clinical studies in which the emollient was used as the active ingredient (either acting as a skin moisturizer or as a skin elasticity provider; both emollients were lipophilic);
2. Skin penetration studies in which a hydrophilic or a lipophilic active ingredient were included in formulations using emulsifiers that formed either liquid-crystalline emulsions or not.

Results:

In the clinical trials in which 36 formulations were tested for either skin moisturization (18 formulations; 9 containing a good moisturizing emollient and another 9 containing a poorly moisturizing emollient) or skin elasticity (another 18 formulations; also 9 with a good elasticity-providing emollient and another 9 with a poor elasticity-providing emollient). All formulations were applied for 6 hours. Formulations with a liquid-crystalline structure gave better skin moisturization or skin elasticity as measured by the Corneometer or Dermal Torque meter, respectively (see Figure 2). From this, we concluded that these formulations deliver more of the lipophilic moisturizer or elasticity-provider into the (epi)dermal layers of the stratum corneum at 6 hours.

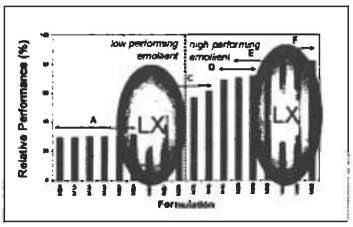

Figure 2: (Epi)dermal delivery (and thus moisturization) is better from liquid-crystalline formulations.

In the skin delivery studies, the total skin penetration of the lipophilic octadecenedioic acid was the same after 24 hours from a liquid-crystalline and a non-liquid crystalline formulation. However, while the dermal delivery was high and the transdermal delivery low from the non-liquid crystalline formulation, this was reversed for liquid-crystalline formulation, suggesting the skin penetration of lipophilic ingredients to be faster from liquid crystalline systems (see Figure 3). In contrast, the total skin penetration of the hydrophilic propagermanium increased significantly from a liquid-crystalline formulation but the transdermal delivery was low from both formulations types. From this, we conclude that the skin

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penetration process is prolonged (hence more penetration) for hydrophilic active ingredients but enhanced for the lipophilic active ingredients (hence more transdermal delivery).

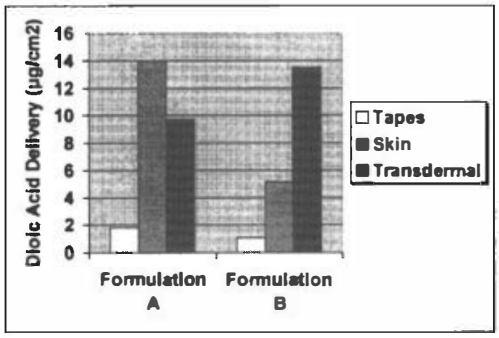


Figure 3: Skin delivery of the lipophilic octadecenedioic acid is faster from a liquid-crystalline formulation (B) than from a non-liquid crystalline formulation (A).

Discussion:

At first glance, the results seem somewhat inconsistent but sense can be made if the factor time is brought into consideration. For hydrophilic active ingredients, the water in liquid-crystalline formulations is structured (see b in Figure 4) and as a consequence, it evaporates less, keeping the active ingredient in solution and therefore to penetrate for longer. This explains the increased penetration of hydrophilic active ingredients from liquid-crystalline formulations at 24 hours.

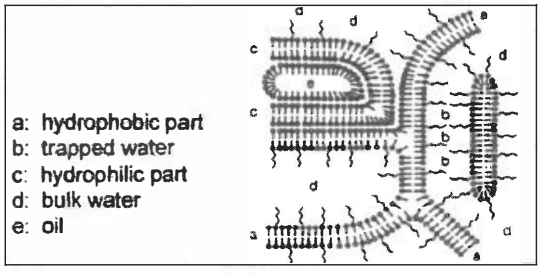
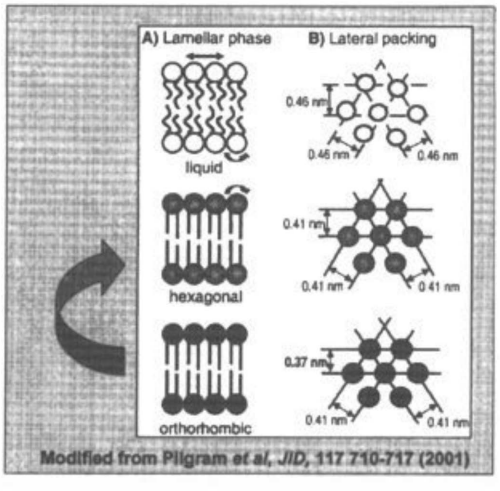


Figure 4: Hydrophilic chemicals remain solubilized longer in trapped water within liquid-crystalline emulsion structures



For lipophilic active ingredients, we postulate a shift in the skin lipid packing from orthorhombic to hexagonal (see Figure 5). This will result in an increased skin permeability of active ingredients of all polarities, explaining the observed increase in (epi)dermal delivery of the moisturizing and elasticity-providing emollients at six hours and the increased transdermal delivery of the lipophilic active ingredient octadecenedioic acid at 24 hours. Transdermal delivery of the hydrophilic propagermanium had indeed increased but was still low, suggesting that for hydrophilic ingredients the time-extension is more prominent than the increase in skin permeability.

Figure 5: A shift from orthorhombic to hexagonal skin lipid lacking will increase the skin permeability of all active ingredients.

Liquid-crystalline emulsion structures may therefore be used when increased efficacy (and thus increased skin delivery) is needed for hydrophilic active ingredients and when faster efficacy (and thus faster delivery) is needed for lipophilic active ingredients. In the latter case, however, it may be necessary to change from single daily dosing to multiple daily dosing to maintain clinical efficacy over a prolonged period of time.

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DEVELOPMENT OF THE NOVEL PLANT-DERIVED LANOLIN SUBSTITUTE AND ITS COSMETIC APPLICATIONS

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Abstract

We have developed Bis-behenyl/isostearyl/phytosteryl dimer dilinoleyl dimer dilinoleate (oligomer ester) as a novel plant-derived lanolin or adsorption refined lanolin substitute with very pale color (GH=1), low odor, and excellent stability. It is an oligomer type phytosterol ester in a paste form synthesized only from plant-derived materials. This oligomer ester has excellent lanolin-like properties such as high water holding capability (200%), excellent moisturizing effect in human use tests, excellent gloss in makeup products (superior to lanolin and adsorption refined lanolin) coming from its high refractive index (approx. 1.475 at 60 °C), high pigment dispersing ability, excellent foam-stabilizing ability and moisturizing effect in cleansing products and shampoos, emulsion stabilizing effect, skin affinity, etc. And this oligomer ester is much more stable against oxidation than lanolin and adsorption refined lanolin in CDM test. So this oligomer ester is useful for make-up products, cleansing products, skincare products, and haircare products.

Introduction

Lanolin has been used as a cosmetic ingredient for a long time because of its good properties such as water holding capability, skin affinity, gloss coming from high refractive index, pigment dispersing ability, and so on. But lanolin has also some problems (not so good color, odor, and oxidation stability). The properties of lanolin are mainly due to its characteristic composition profiles, that is, complicated mixed esters consisted of extremely many kinds of fatty acids and alcohols, including sterols and branched fatty acids / alcohols, and high molecular weight.

In this investigation, we have attempted to develop a new lipid having similar properties to lanolin (and adsorption refined lanolin) only from plant-derived raw materials. Therefore we have designed in consideration of the above composition profiles of lanolin and synthesized Bis-behenyl/isostearyl/ phytosteryl dimer dilinoleyl dimer dilinoleate (oligomer ester; Figure 1) from hydrogenated dimer acid, dimerdiol, behenyl alcohol, isostearyl alcohol, and phytosterol as a novel plant-derived lanolin without the problems of lanolin [1].

R3-OCO-R1(-COO-R2-OCO-R1)n-COO-R3

R1: Dimer acid residue, R2: Dimerdiol residue. R3; Phytosterol, Behenyl / Isostearyl alcohol residue

Figure 1: Molecular structure of the oligomer ester

Physical properties of the oligomer ester

Bis-behenyl/isostearyl/ phytosteryl dimer dilinoleyl dimer dilinoleate (oligomer ester) has excellent lanolin-like properties. It's a paste ester with a melting point near the body temperature (approx. 38 °C) and a thixotropic flow like lanolin. It also has high water holding capability (200%, Figure 2), high refractive index (approx. 1.475 at 60 °C) and excellent spread gloss in model lipsticks (superior to lanolin and adsorption refined lanolin, Figure 3), high pigment dispersing ability (very low flow point and wet point, , Figure 4), and good skin affinity, which are very similar to lanolin. On the other hand, the color (GH=1) and odor of the oligomer ester are superior to those of lanolin. And the oligomer esters is much more stable against oxidation (induction period >48hr) than lanolin and adsorption refined lanolin (<8hr)) in CDM test (temp:120°C, air:20L/hr, Figure 5).

Features of the oligomer ester in cosmetic products

The oligomer ester has excellent effects in cosmetic products. The oligomer ester showed excellent moisturizing effect in human use tests (in vivo water sorption-desorption test [2]; after 4-week-application of the oligomer ester cream, skin conductance (approx. 2.5 times, Figure 6) and water holding capacity were extremely improved and skin barrier function was improved), emulsion stabilizing effect, and excellent foam-stabilizing ability (superior to lanolin, Figure 7) and moisturizing effect (skin conductance) in cleansing products and shampoos.

So this oligomer ester is considered (to be useful) for make-up products (especially high gloss

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lipsticks and moisturizing foundations), cleansing products, skincare products (especially emollient creams and UV care cosmetics), and haircare products (especially shampoo and rinse-off and leave-on hair conditioners for damaged hair).

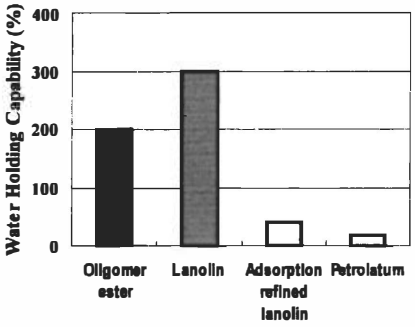

Figure 2: Water holding capability

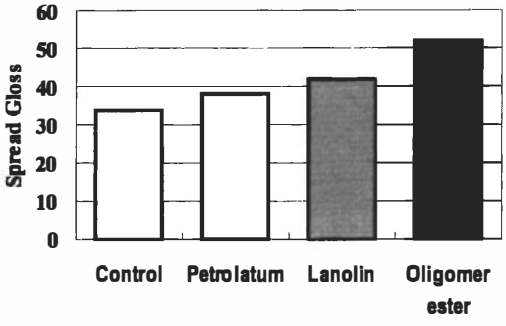

Figure 3: Spread gloss of model lipsticks

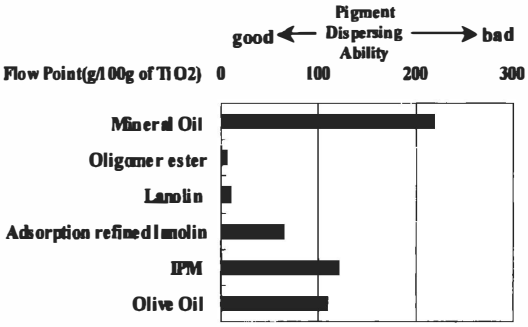

Figure 4: Dispersing ability to Titanium dioixide

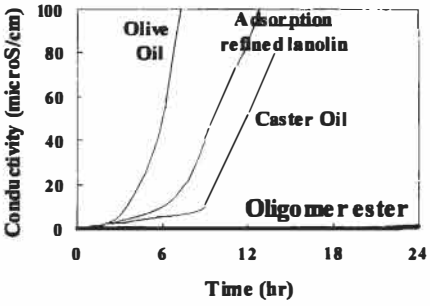

Figure 5: Oxidation stability (CDM Test)

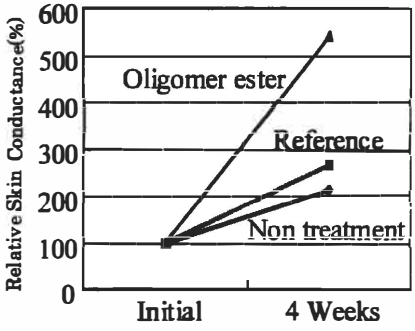

Figure 6: Skin conductance in human use test (Skincare cream)

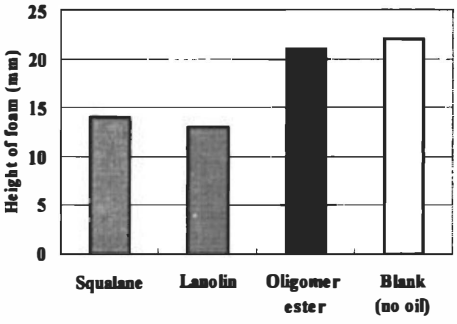

Figure 7: Foam stabilizing ability (Body soap)

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PEPTIDES - MORE THAN ANTI-AGING: A NOVEL ANTIMICROBIAL PEPTIDE FOR COSMETIC APPLICATIONS

Durant Scholz, C. Murphy and R. Rivera

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A casual survey of the latest edition of the CFTA dictionary shows a listing of more than 100 different peptides. These range from small peptides with well established sequences to less well defined materials isolated from grains or soy, although these are not well differentiated from simple hydrolysates. A clear majority of these products are designed to support some type of anti-aging claim although the proposed mechanisms vary considerably. But that of course, is clearly what the consumer has come to expect from peptides. It may well be that peptides, like elastomers before them have become the next AHA, a raw material that is *de rigueur* in modern cosmetics.

In molecular biology peptides function ubiquitously as cellular messengers. It is this role that much of the focus of research within the Cosmetic Industry has focused on. The role we will be looking at revolves around the ability of certain peptides to function as a component of an organism's innate immune system. Research in both food and medicine has flirted with this area for over 50 years, although it has only been the past 5 years that have brought a dramatic innovation. Much of this interest was driven by the high degree of sequence homology between antimicrobial peptides (AMP) across species lines.

Our particular interest was sparked by the lack of incidence on the Korean peninsula relating to the SARS outbreak. It was postulated at that time, that consumption of Kimchi, a form of cabbage fermented with *Leuconostoc sp.*, was responsible for the apparent disease resistance. Regardless of the veracity of this hypothesis we began looking for AMP produced by the various species of *Leuconostoc* typically present in Kimchi. Our research ultimately focused on *Leuconostoc kimchii.*

A BLAST (Basic Local Alignment Search Tool) search of the genome revealed sequences for several potential antimicrobial peptides. We were able to isolate the peptides from culture and identify the corresponding genomic sequence. The genes were isolated and inserted into a tagged expression vector in E. coli. Once the expression of the recombinant peptide was confirmed the expressed peptides where fractionated and purified. The resulting peptides where screened for their antimicrobial efficacy. One of the candidate peptides PF1056 showed excellent stability and strong antimicrobial efficacy making it an ideal candidate for cosmetic application. PF1056 was tested for kill rate, MIC and its ability to preserve cosmetic systems. PF1056 is a cationic peptide made up of 29 amino acids, with a molecular weight of approximately 3,000.

The MIC studies where conducted as follows. Inoculate 5 ml LB in tubes with test strains from LB plates and incubate overnight at 37°C on a shaker (180 rpm). Make serial dilutions of test samples at 10 times the required test concentrations. Dissolve test samples in distilled H_2O at 20 times the required maximal concentration 640, 320, 160...2.5 µg/ml. Dilute overnight bacterial cultures in LB to give 1 x 10^6 cfu/ml (Yeast 1 x 10^5 cfu/ml). Dispense 100 µl of bacterial suspension in each well from column 1 to column 11. Do not add bacteria to column 12, and instead dispense 100 µl of LB (sterility control and blank for the plate scanner). Add 100 µl of PF1056 (10x) to each well from column 1 to column 10 (column 11 is a control for bacteria alone, with no sample). Incubate the plates at 37°C for 18-24 hours. Plates are checked again at 40-48 hours. When satisfactory growth is obtained (18-36 hours) scan the plates with an ELISA reader (or read by eye). MIC can be taken as the lowest concentration of drug that reduces, by more than 50% or 90% for MIC_{50} or MIC_{90} respectively. **[Figure 1.]**

Kill rates were performed using a standard agar dilution method. **[Figure2.]** To evaluate temperature stability a 1% solution of PF1056 was held at either 37°C or 100°C for a period between 20 and 120 minutes. The heat treated solution was then used to conduct a zone of inhibition study. The study confirmed that PF1056 is unaffected by heat treatment within the range tested. **[Figure 3.]** A fresh 1% solution of PF1056 was prepared and divided in to aliquots. The aliquots were adjusted to pH ranging from 3 – 10 using either citric acid, or sodium hydroxide. **[Figure 4.]** Again zone of inhibition studies were conducted to confirm activity. Activity was seen at a pH range of 3 – 5 and again at a pH of 8 indicating that the material would be suitable in most skin care and some surfactant systems.

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A simple lotion formula was preserved with 1% PF1056 and challenge tested using *S. aureus, B. subtilis, E. coli, P. aeriginosa, C. albicans, and A. niger* [**Figure 5.**]. Plates were counted at 24 hours, 48 hours, and 21 days. [**Figure 6.**] The results clearly demonstrate the potential PF1056 as a viable part of a cosmetic preservative system.

Figure 1

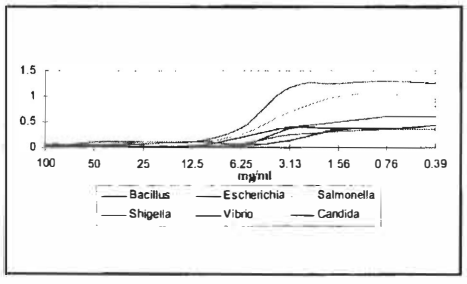


Figure 2

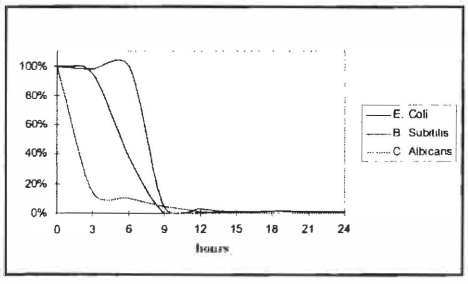
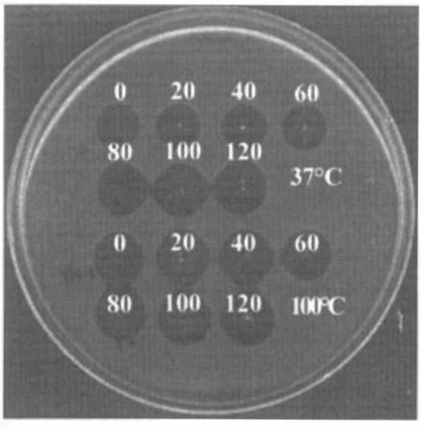


Figure 3

Figure 4



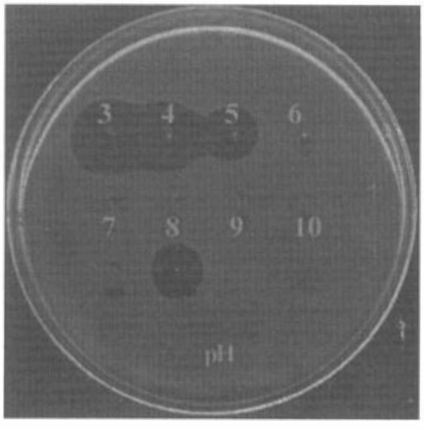


Figure 5

Figure 6

Test Formulation

Water	91.2 %
Yeast Extract	2.8 %
Propylene Glycol	5.0 %
PF1056	1.0 %

Challenge Test

Organism	24 Hour	48 Hour	21 Day
Gram +	<10	<10	<10
Gram -	<10	<10	<10
Yeast	<10	<10	<10
Mold	8×10^1	<10	<10

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4-(1-PHENYLETHYL) 1,3-BENZENEDIOL: A NEW HIGHLY POTENT LIGHTENING AGENT

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Introduction

There is an increasing worldwide demand for skin lightening active ingredients. Whereas a pale skin is the beauty ideal in Asian countries, Caucasian skin types from Europe and the US aim to treat pigment spots. Current skin lightening actives such as Kojic acid, Arbutin or Ascorbic acid derivatives have several disadvantages regarding their safety or stability. Since nature is without any doubt an inexhaustible source of inspiration for new actives for cosmetics, we recently focused within a continuously running natural product research program on different wood extracts and pure isolates, obtained thereof. The goal was to evaluate their tyrosinase inhibitory activity and to identify a new skin lightening agent, lacking those negative side effects described above.

Materials and Methods

Origin of test material: Phenolic compounds with different substitution patterns were either isolated from plant extracts by preparative liquid chormatography or they were synthesized.

Mushroom Tyrosinase Assay: Tyrosinase reactions were performed in a 96well microplate with 66.7 mM phosphate buffer (pH 6.8) containing 50 u/ml mushroom tyrosinase (EC.1.14.18.1, Fluka Chemie GmbH, Buchs, Switzerland) and test compounds. After pre-incubating at 37°C for 10 min, L-3,4-dihydroxyphenylalanine (L-DOPA) was added to the mixture and the formed dopachrome was measured photometrically at 475 nm. The inhibitory effect of the test samples was expressed relative to the control and IC50 (50 % inhibitory concentration) values were calculated. Kojic acid was used as a reference.

In vitro Toning Assay with B16V Mouse Melanoma Cells: B16V cells were seeded into 96well microplates. After adhesion (24 h) the medium was replaced by freshly prepared solutions of the test compounds at non cytotoxic concentrations (solvent: cell culture medium containing 10 nM α-Melanocyte Stimulating Hormone). After incubation for another 96 h, melanin was extracted with NaOH and the absorption at 400 nm was measured.

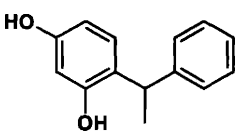
In vitro Toning Assay with Pigmented 3D Epidermis Models: Freshly prepared solutions of the test compounds (solvent: PBS) were applied at non cytotoxic concentrations on the top of MelanoDerm™ MEL-300-B models (skin type IV). The test compounds were reapplied daily. After overall incubation time of 7 and 19 days, respectively, the melanin was extracted with soluene and the absorption at 400 nm was measured.

In Vivo Lightening Efficacy was studied in a human model on Asian skin. Test products and placebo formulation were applied 2 times daily. Subjects were requested to avoid any UV exposition during the test period. Measurement of the lightening efficacy was done by chromametry and visual assessment at t = 0 and after 28 days.

Results and Discussion

Within our natural products screening the tyrosinase inhibiting activity of „Scotch Pine" (*Pinus sylvestris*) heart wood extracts and pure isolates thereof attracted our specific interest. From this extract we isolated stilbene derivatives like Pinosylvin-3-O-methylether and Pinosylvin. Since we observed that such stilbene derivatives are fairly unstable under light condition, more stable partially hydrogenated derivatives like Dihydropinosylvin-3-O-methylether and Dihydropinosylvin, both also occurring in trace amouts in pine heart wood, as well as a couple of nature-derived substances with very similar structure like 4-benzyl-1,3-benzenediol, 2-(1-phenylethyl)1,3-benzenediol and 4-(1-phenylethyl)1,3-benzenediol were synthesizd. The inhibitory activity of all compounds was measured with the same mushroom tyrosinase assay and their IC_{50} values relative to Kojic acid were calculated. Several compounds like Pinosylvin-3-O-methylether, Dihydropinosylvin-3-O-methylether, 4-benzyl-1,3-benzenediol and 2-(1-phenylethyl)1,3-benzenediol showed only weak to moderate tyrosinase inhibitory activity. 4-(1-phenylethyl)1,3-

Figure 1: Structural formula of 4-(1-phenylethyl)1,3-benzenediol



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benzenediol (Fig. 1) was by far the most active compound within this compound class. It exhibited an IC_{50} of 0.50 µM, thus reducing tyrosinase activity approximately 22 times more effectively than Kojic acid with an IC_{50}= 11.05 µM under identical test conditions. Because of its excellent tyrosinase inhibitory activity, further detailed in vitro studies in melanocyte cultures and on 3D skin models as well as first human in vivo studies were performed with 4-(1-phenylethyl)1,3-benzenediol. In the cellular lightening assay on B16V mouse melanoma cells, a pronounced efficacy of 4-(1-phenylethyl)1,3-benzenediol was found. It was by far the most potent inhibitor of melanin synthesis with an IC_{50} of 2.1 µM, whereas the IC_{50} of ß-arbutin and Kojic acid was 67 µM and 440 µM, respectively, under identical test conditions. This means that 4-(1-phenylethyl)1,3-benzenediol was approx. 200 times stronger than Kojic acid in the cellular assay. In the pigmented 3D skin model, 4-(1-phenylethyl)1,3-benzenediol reduced the melanin content of pigmented 3D epidermis models at least 10 times more efficiently than Kojic acid within a period of 19 days (Fig. 2).

To confirm that 4-(1-phenylethyl)1,3-benzenediol sufficiently penetrates into skin and is effective also under human *in vivo* conditions a clinical study was performed with Asian subjects during a duration period of 4 weeks (Fig. 3). Product GS05048SL-A and –B (0.5 % 4-(1-phenylethyl)1,3-benzenediol each) efficiently lighten the natural tone of Asian skin *in vivo* after 2x daily treatment for 28 days. 0.5 % 4-(1-phenylethyl)1,3-benzenediol is more efficient than 1.0 % Kojic acid. A more pronounced effect is achieved when 4-(1-phenylethyl)1,3-benzenediol is directly incorporated into an aqueous gel formulation with low oil phase content (GS05048SL-A). Pre-solubilising 4-(1-phenylethyl)1,3-benzenediol in a formulation with high oil phase content (GS05048SL-B) was less effective. Despite the considerable concentration gradient between the applied formulation and the skin itself, 4-(1-phenylethyl)1,3-benzenediol is obviously only poorly released from the oil phase because of it high lipophilicity

Conclusion

Systematic screening of plant extracts, isolated natural products and nature-derived synthetic derivatives thereof for potential skin lightening activity showed, that 4-(1-phenylethyl)1,3-benzenediol, a dihydroxylated diphenylmethane derivative, possesses potent tyrosinase inhibitory activity. Further investigations on the lightening activity in a cellular lightening assay (B16V mouse melanoma cells) and on pigmented 3D skin models (MelanoDerm™ skin type IV). delivered the unequivocal proof *in vitro*, that 4-(1-phenylethyl)1,3-benzenediol is one of the most potent lightening agents ever reported. First studies on Asian skin confirmed that it also shows good lightening activity in a human *in vivo* situation.

Figure 2: Lightening activity of 4-(1-phenylethyl) 1,3-benzenediol on pigmented 3D epidermis models (MelanoDerm™, skin type IV); photos were taken after 19 days of daily treatment with Kojic acid and 4-(1-phenylethyl) 1,3-benzenediol (Bio377), respectively.

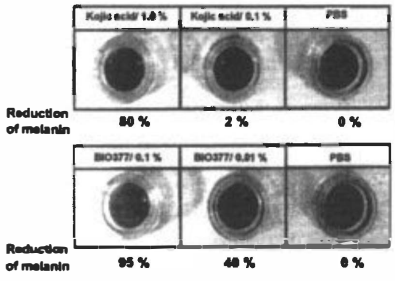
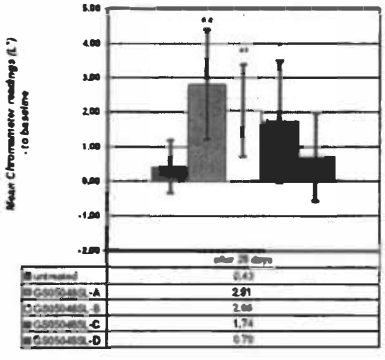


Figure 3: In vivo efficacy of 4-(1-phenylethyl) 1,3-benzenediol on non-irradiated skin (mean chromameter readings to baseline after 28 days); 4 cosmetic formulations were studied: GS05048SL-A (0,5% 4-(1-Phenylethyl)1,3-benzenediol); GS05048SL-B (5% of a 10% solution of 4-(1-Phenylethyl)1,3-benzenediol in neutral oil); GS05048SL-C (1% Kojic acid); GS05048SL-D (placebo formulation).



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IMPROVING EFFICACY OF SKIN LIGHTENING PRODUCTS BY USING MULTIPLE PIGMENTATION INHIBITION MECHANISIMS

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Summary: The efficacy of skin lightening products containing multiple pigmentation inhibition mechanisms was examined via in-vitro and in-vivo studies. The results were compared with that of formulas containing only a single inhibition mechanism. The multiple mechanism products showed higher reduction in total melanin than the single mechanism ones in the in-vitro efficacy studies where cultures of reconstructured human epidermis were used. In-vivo studies confirmed the advantage of such an approach. More than 30% reduction in mottled pigmentation and 50% reduction in dark skin tone were achieved after a 12-week use period. This study shows that multiple inhibition mechanism improves the skin lightening efficacy of a product.

Introduction: Skin lightening products take a large market share in Asian countries. Consumers continue to demand for more efficacious products which often marketed under an OTC-equivalent product category, such as quasi-drug cosmetics in Japan, special use cosmetics in China, and functional cosmetics in Korea. The levels and functions of ingredients under this category are usually regulated. To increase product efficacy, utilization of multiple pigmentation inhibition mechanisms provides a way [1]. Melanogenesis of the skin involves multiple pathways and skin lightening can be achieved through many mechanisms other than tyrosinase inhibition [2]. In this study, we formulated products with ingredients exhibiting various pigmentation inhibition properties such as tyrosinase inhibition, melanosome transfer inhibition, α-MSH inhibition, eumelanin-pheomelenin ratio alteration, and existing melanin removal. In-vitro and in-vivo studies were conducted to confirm the efficacy.

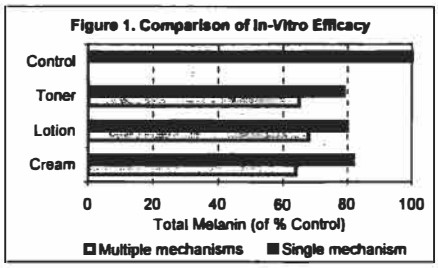
Material and Methods: Tyrosinase activity assay [3] was used to screen melanin inhibition activity of various ingredients. In-vitro efficacy was tested using a 3-D model of reconstructured human epidermis with melanocytes (Melanoderm from MatTek). The samples of skin model were incubated with various ingredients or products three times a week for two weeks. Total melanin, surface coloration, cell liability, and microscopic images were examined after that [4]. In-vivo studies were conducted via clinical tests in an independent testing lab on the face of 60 female Asian subjects. Skin lightening parameters such as skin clarity, skin tone, and mottled pigmentation were clinically graded based on a 10 point scale. Color pictures were taken at the time of evaluation.

Results: Various ingredients were screened for pigment inhibition activity. Some significant results are listed in Table 1 where tyrosinase inhibition activity (column 3) was compared with total melanin and surface coloration results (columns 4 & 5). Notice the trend in column 3 do not translate into column 4 indicating some ingredients worked under mechanisms other than tyrosinase inhibition. For instance, while citrus unshiu extract showed a much lower tyrosinase inhibition than bearberry extract and the botanical blend, their levels of inhibition in melanin production in 3-D skin model were comparable. For wheat germ extract, it had a 10% inhibition in tyrosinase activity. However, it inhibited about 20% melanin production in 3-D skin model. Its coloration score was also very low indicating not much eumelanin was produced on the surface of the skin model. These results supported literature and vendor data, as citrus unshiu extract is known to inhibit melanin production in addition to tyrosinase inhibition. Wheat germ extract is believed to switch the melanin production from eumelanin (a dark/brown melanin) to pheomelanin (a light/yellow melanin) while the content of lectins in the material inhibits transfer of melanosomes from the melanocytes to the keratinocytes [5].

Comparative efficacy data were obtained when incorporating ingredients with various pigment inhibition mechanisms into some cosmetic formulas. Their efficacy of melanin inhibition was measured against the formulas with single inhibition mechanism. In both systems a 3% magnesium ascorbyl phosphate was used to establish an OTC-equivalent status. Figure 1 shows the total melanin content of the 3-D skin models after treated with various products for two weeks. It is seen that the single mechanism formulas had an average reduction in total melanin of

Table 1

	Conc.	Tyrosinase Inhibition (%)	Total Melanin (% Control)	Coloration (1-5 scale)
Control (culture medium)	--	--	100.0	3
Mg-Ascorbyl Phosphate	3%	--	53.0	2
Na-Ascorbyl Phosphate	3%	--	49.0	2
Bearberry Extract	2%	99.7	62.0	2
Botanical Blend 4	2%	91.0	68.0	3
Botanical Extract 2	0.1% solids	70.3	45.0	2
Raspberry Extract	2%	39.3	87.0	4
Citrus Unshiu Extract	2%	24.2	82.0	1
Wheat Germ Extract	2%	11.4	81.0	1



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about 20% whereas the multiple mechanism formulas achieved about 35%.

Figure 2 shows the images of surface melanin on the 3-D skin models where (A) is the control, (B) from a single mechanism formula, and (C) from a multiple mechanism formula. We see that much less melanin is shown on (C) than on (B) which indicates the formula with multiple mechanisms is more efficacious than that of a single one.

Figure 2. Efficacy - Image of Surface Melanin on Skin Model
(A) (B) (C)

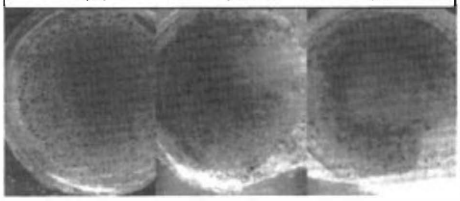
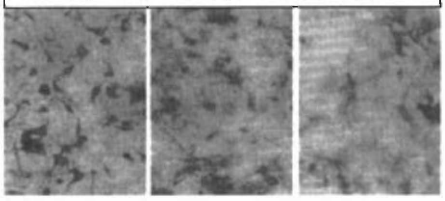


Figure 3. The Melanocytes at the Bottom of 3-D Skin Model
(A) (B) (C)



The morphology of the melanocytes is shown in Figure 3. The melanocytes treated with the control (A) were full of melanosomes. Much less melanosomes and melanocytes were seen in the product treated samples. The shape of the melanocytes became thinner, and the color of their dendrites was more transluscent. The multiple-mechanism sample (C) had clearly less melanin and melanocytes than the single-mechanism one (B) indicating a better skin lightening efficacy. The in-vitro results were then confirmed through a couple of clinical studies. As shown in Figures 4 – 6, the multiple mechanism formula out performed the single mechanism formula in a 12-week study in the U.S., and a 4-week study in Japan. Figure 7 shows the before and after photos comparing the overall skin conditions, such as mottled pigmentation, skin tone and clarity, of a panelist's face.

Figure 4. Invivo Efficacy in U S - Mottled Pigmentation

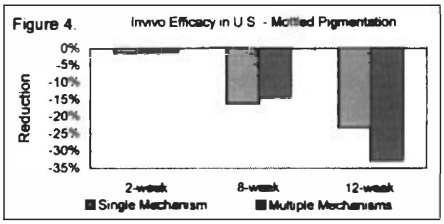


Figure 5. Invivo Efficacy in U S.- Dark Skin Tone

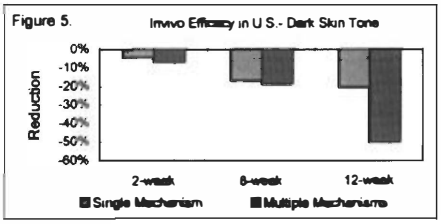


Figure 6 Invivo Efficacy Study in Japan (4 weeks)

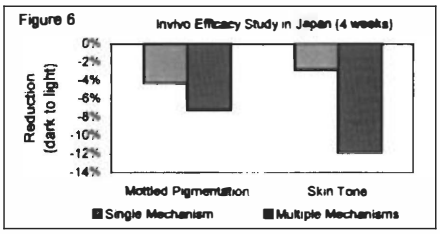
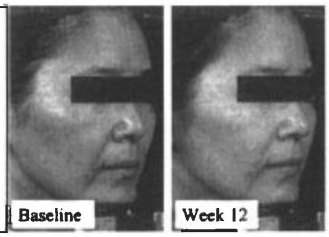


Figure 7. Sample pictures from a 12-week in-vivo study in the U.S.



Discussion: It was noticed from the in vitro study that single ingredient performed better than a complete formula. We believe the phenomenon was caused by the interactions among various ingredients as we had seen certain ingredients promoted pigmentation in the in-vitro study and we might have used some ingredients in a formula for other functions without knowing their counteractive effects in pigment inhibition.

Conclusion: Formulas with multiple pigment inhibition mechanisms show better efficacy than single mechanism ones in our in-vitro and in-vivo studies. The combined action is clearly better than a single action. This approach provides a meaningful way to increasing performance of skin lightening products in the OTC-equivalent category.

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BOTANICALS FROM TRADITIONAL CHINESE MEDICINE (TCM) AND THEIR POTENTIAL AS NATURAL ANTI-IRRITANTS IN COSMETIC APPLICATIONS

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Introduction

Many plants are known that are traditionally used for their anti-inflammatory activity. In most cases, the evidence for the anti-inflammatory activity is derived from animal models or immunocompetent cells but efficacy proof on human skin cells is scarce. In skin, interleukin-1 (IL-1α) is a key mediators in the inflammatory process. Increased cutaneous levels of IL-1α are found in conditions with a weakened epidermal barrier such as aged skin, dry skin or sensitive skin as well as in photodamaged skin [1-4]. However, specific IL-1α inhibitors are rare. We have evaluated the IL-1α inhibitory efficacy of plant extracts from traditional chinese medicine (TCM) on human keratinocytes. Since reactive oxygen species are a major trigger in the inflammatory cascade, we have also investigated whether the radical scavenging activity of the extracts significantly contributes to their anti-irritant activity.

Materials and Methods

IL-1α Assay: HaCaT keratinocytes were seeded into 96well microplates and grown to 90-100 % confluency. The medium was exchanged against solutions containing the test compounds. After 1 h, IL-1α biosynthesis was stimulated by addition of 0.4 µM A23187 (Sigma, Taufkirchen, Germany). After a further 6 h, the cells were lysed with 1% Triton X 100 in PBS and the IL-1α content was measured with the Human IL-1α ELISA Kit from Perbio Science (Bonn, Germany). Dexamethasone was used as a reference (1 µM = 43% IL-1α inhibition).

LDH Assay: Lactate dehydrogenase (LDH) activity in the supernatant was determined by use of the Cytotoxicity Detection Kit from Roche (Mannheim, Germany).

ABTS Assay: Samples were incubated for 10 min at 30°C with the 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonate (ABTS) radical cation which was prepared by reaction of ABTS with potassium persulfate. The degree of decolorization corresponds to the radical scavenging activity and was determined spectrophotometrically at 734 nm .

Results and Discussion

For measurement of IL-1α the human keratinocyte cell line HaCaT was used since these cells were easier to handle than primary keratinocytes. To assure that the enhanced levels of IL-1α were not due to cytotoxicity, a LDH assay with the supernatant was performed in parallel to the anti-irritant assay.

Japanese honeysuckle flowers (*Lonicera japonica*), kudzu roots (*Pueraria lobata*) and Japanese pagoda tree flowers (*Sophora japonica*) were found to possess considerable anti-irritant activity. At 1 %, the extract preparations containing 3% of dry extract (trade name: Extrapone®) inhibited IL-1α by 40 % (Honeysuckle) and 29 % (Pueraria, Sophora), respectively. Additionally, 0.1 % of the extracts also exhibited potent radical scavenging capacity in the cell-free ABTS assay with a maximum antioxidant capacity of 25 % (Honeysuckle), 81 % (Pueraria) and 22 % (Sophora), respectively.

To elucidate the respective active principles of the TCM extracts, phytochemical characterization was performed by HPLC-DAD-MS analysis. As major UV-detectable components chlorogenic acid (0.08%), puerarin (0.17%), and rutin (0.10%), respectively were identified. However, the anti-irritant and radical scavenging activity of the single compounds and the extracts did not correlate. Only in the case of Sophora the radical scavenging activity could be fully ascribed to its major component rutin.

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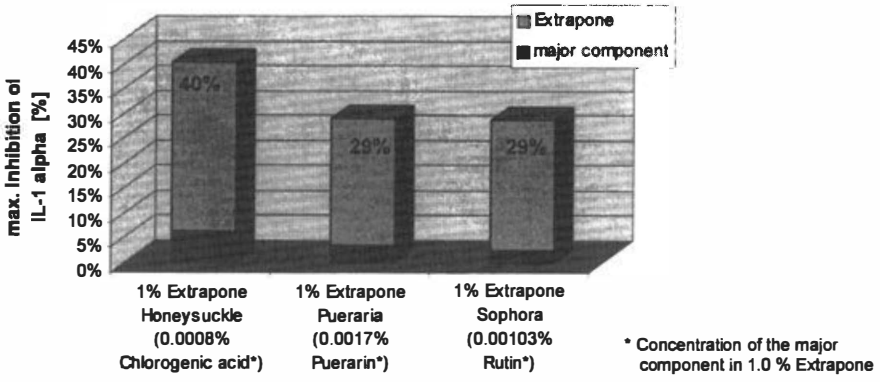

Figure 1: IL-1alpha inhibition of TCM Extracts and the respective major components.

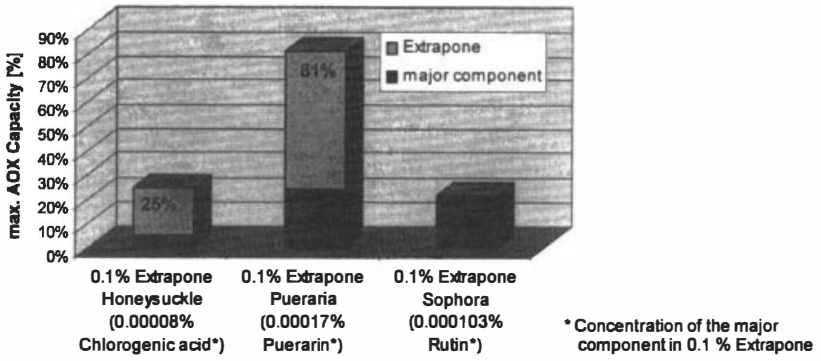

Figure 2: Antioxidant capacity of TCM Extracts and the respective major components.

Conclusion

Extracts from the TCM plants Japanese honeysuckle (*Lonicera japonica*), kudzu (*Pueraria lobata*) and Japanese pagoda tree (*Sophora japonica*) exhibit potent anti-irritant activity in human epidermal keratinocytes. The anti-irritant activity of these extracts does not correlate with their radical scavenging activity. Moreover, only a small part of the anti-irritant and anti-oxidant activity of the extracts could be ascribed to the respective major ingredients. Thus, the presence of several ingredients in a botanical extract is apparently essential for optimal efficacy.

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SALICYLIC ACID PROTECTS THE SKIN FROM UV DAMAGE

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Abstract
Aspirin (acetyl salicylate) has long been used as an analgesic. Salicylic acid has previously been reported to have anti-inflammatory properties. These activities include inhibiting activity of cox-1, cox-2, and NF-kb. In addition, salicylic acid has also been shown in some systems to induce Hsp70. We have demonstrated that salicylic acid inhibits UVB-induced NF-kb activation in keratinocytes. In addition, salicylic acid was also found to induce Hsp 70 in keratinocytes and increase keratinocyte survival to UVB toxicity. In living skin equivalents, salicylic acid was found to reduce UVB induced sunburn cell formation, as well as increase the removal of UVB induced TT dimer formation in living skin equivalents. Given these protective properties of salicylic acid, we propose the use of salicylic acid as a topical therapeutic to protect the skin from sun damage.

Introduction
Willow Bark has been used for centuries in Europe and China, and is still used today for its multiple medicinal properties. The medicinal ingredient, salicylic acid has long been used in modern medicine, initially as an analgesic. Today, aspirin (acetyl salicylate) is taken as an analgesic, anti-inflammatory, blood thinner, and as preventative medicine for heart disease. Salicylic acid has been reported as a cox-2 inhibitor (Wu, 2003) as well as an inhibitor of NF-kb activity (Kwon, 2003, and Constanzo, 2003), which would explain in part, its analgesic properties. In skin care, salicylic acid is used for acne treatment and for skin desquamation. However, it has also been reported that salicylic acid activates the binding of the HSF-1 transcription factor to the heat shock response element upstream of the Hsp70 gene in mammalian cells (Jurivich, 1992) Recently, it was reported that sodium salicylate was found to induce heat shock proteins in mammalian cells (Ishihara, 2003). As heat shock proteins such as Hsp70 have been shown to be cytoprotective, it is likely that salicylic acid may be useful as a protective agent in skin.

Methods
NF-kb activation assay:
Normal Human Epidermal Keratinocytes (NHEK) were grown on 100mm plates to 50% confluence in the absence of hydrocortisone. These NHEK were then treated with 0, or 1mM salicylic acid for 6hrs. The keratinocytes were then treated with 0-25mJ/cm2 UVB. NF-kB p65 was isolated from NHEKs with the Trans-AM NF-kB p65 kit (Active Motif). The kit contains an ELISA plate with oligonucleotides containing an NF-kb consensus-binding site for sequestering NF-kB present in NHEK nuclear extracts. Detection of the NF-kb protein is via a primary antibody and conjugated secondary antibody. Following the addition of substrate, the enzymatic reaction is allowed to proceed for up to 10 minutes before measuring the absorbance on a spectrophotometer at 450nm.

Hsp70 protein determination:
NHEKwere grown to 75% confluency in 6 well plates before being treated with different doses of salicylic acid (0-100mg/ml). These treatments were carried out for 24 hours. Following the post incubation, the keratinocytes were harvested and pelleted. The Hsp70 ELISA kit from StressGen was used to quantify the levels of Hsp70 in the NHEK samples.

UVB Viability Assay:
NHEK were grown to 75% confluency in 6 well plates before being treated with different doses of salicylic acid (0-100mg/ml). Follwing a 6 hour pre-incubation, the NHEK were exposed to 30, 60, and 90mJ/cm2. The MTS survival assay was done 18hrs post UVB treatment.

Host-cell reactivation assay:
Fibroblasts from a 31 year-old donor (ATCC) were incubated with liposomes containing the pSEAP DNA reporter (SV40 promoter fused to an alkaline phosphatase reporter). Cells were plated in 24 well plates and grown for 24 hours prior to exposure with liposomes loaded with reporter DNA. Fibroblasts were treated for 24hrs prior to transfection and 48hrs after transfection with salicylic acid. The DNA reporter-incorporated liposomes were then transfected into the fibroblasts. DNA damage was inflicted with UVB irradiation equivalent to 200, 400, 600, 800, 1000 or 1500 mJ/cm2. The positive control cells were treated with liposomes containing reporter DNA, but not exposed to UVB. Untreated cells did not receive reporter DNA. The vector-encoded alkaline phosphatase is heat stable at 65oC. To each well, 97 ml of assay buffer was added and incubated for 5 min at room temperature prior to adding 3 ml of 1mM MUP. The plate was incubated for 60 min in the dark at room temperature before measuring the fluorescence (excitation: 360 nm and emission: 460 nm).

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UVB-induced Sunburn cell formation:
Excised portions (8mm) were taken from living skin equivalents (Organogenesis) and cultured over transwell membrane plates. These excised portions were pretreated topically with either distilled water, or salicylic acid (200ug/ml). After the post-incubation, these excised portions were UVB irradiated at 0, 100and 150mJ/cm2. Following a 24hour post-incubation, these skin equivalents were fixed in formalin and stored at -4C. These samples were then sent to Paragon Biotech for H&E staining. Sections were then evaluated using a microscope at 400X magnification. A section was selected from each sample and counts of sunburn cells were made. Sunburn cell levels were determined by %, (number of sunburn cells in a representative field divided by total number of cells in a representative field).

DNA repair of UVB-induced thymine dimers (TT-dimers):
Excised portions (8mm) were taken from living skin equivalents (Organogenesis) and cultured over transwell membrane plates. Excised portions (8mm) were taken from living skin equivalents (Organogenesis) and cultured over transwell membrane plates. These excised portions were pre-treated topically with salicylic acid 200ug/ml (in sterile H2O) for 6 hours. After the post-incubation, these excised portions were UVB irradiated at 0, 100, and 150 mJ/cm2. Following a 24 hour post-incubation, these LSEs were fixed in formalin and stored at -4C. These samples were then sent to Paragon Biotech for immunostaining of TT-dimers. Sections were then evaluated using a microscope at 400X magnification. Representative sections were selected from each sample and counts of cells at 400X magnification expressing TT-dimer staining were calculated. The TT-dimer levels of each section were expressed as number of cells expressing the immunofluorescent TT-dimer antibody tagged stainin that section.

Conclusions
Salicylic acid was found to have anti-inflammatory effect on normal human epidermal keratinocytes. We observed a significant inhibition of UVB induced NF-kb.activation in keratinocytes. In addition, salicylic acid was found to significantly increase the induction of Hsp70 protein in keratinocytes. Salicylic acid was also found to increase keratinocyte cell viability to UVB toxicity, as well as increase DNA repair function of normal human dermal fibroblasts using the host cell reactivation assay. In living skin equivalents, salicylic acid significantly reduce UVB-induced sunburn cell formation and increase DNA repair (removal rate of DNA damage). These results suggest that salicylic acid which has previously been described to have anti-inflammatory properties as well as induce Hsp70 in mammalian cells may have benefits if used topically on the skin. Salicylic acid can act as an anti-inflammatory in the skin as well as increase DNA repair function in skin and may be a valuable topical agent to protect skin from sun damage.

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