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HAIR STYLING & FIXATIVE OVERVIEW

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Introduction

Polymers provide many of the attributes during the styling process, as well as the holding and texture characteristics of the hair. However, the total formulation determines the functionality such as the ease of application, the distribution of polymer on the hair, absence from flaking, added shine, longevity of hold, restylability, and removability from the hair.
The product performance and efficacy is rarely a function of only one active ingredient. The entire formulation and package and the delivery mechanism also contribute to the final characteristic effects developed on the hair. The final hairstyle and styling technique will also determine the type and level of polymers needed in styling and fixative products.

Hair Styling and Fixative products have moved away from products with specific "Hold" properties; i.e. soft/flexible, medium, and hard/ultra hold and are now addressing specific hairstyle solutions. Current worldwide fashion trends, the desire by consumers for healthy hair and style control, and the regulatory environment drive this market.

This presentation will define the Hair Styling and Fixative market, review it's growth over the last 4 years, define what is driving this market segment, review polymer requirements for styling and fixing hair and the polymer product arsenal that formulators have to play with, and lastly review new styling and fixative products introduced in 2004.

Global Styling & Fixative Market Overview:

The global hair styling & fixative market is valued at $7.84 Billion for 2004 and is the 3rd largest market segment behind Shampoos and Hair Colorants.[1] This market segment had a growth rate of 25.5% over the years of 2001- 2004 due to worldwide fashion trends, changes in the regulatory environment, and the development of new product forms to address specific hair styling issues which have changed the landscape of the whole styling & fixative market segment.

Table 1 shows the retail value (rsp) in US $dollars of the Hair Styling & Fixative global market segment versus the Total Hair Care category

Table 1: Retail Value rsp- US$ Mn

Global Market Segment	2001	2002	2003	2004	% Growth 2001-2004
Hair Styling & Fixative	6,268.6	6,526.5	7,244.1	7,864.3	25.45
Total Hair Care	37,885.9	38,914	42,816.7	46,987.7	24.02

Ref: Euromonitor International- 2005

This market growth was driven by: current worldwide fashion trends such as "ready to wear hair", "big hair", and the trend towards healthy looking, manageable hair, whether it is curly or straight.[2] When we look deeper into the market category, we see that the trend towards more natural looking, touchable hair has had the most impact on the Hair Fixative Industry. Hair spray sales continue to decline for the past several years with increased sales in the styling segment in particular the gel/cream/wax market. The hair fixative market continues to be split between stiff-feel and flexible, natural hold. Products that offer multifunctional properties have made the most impact in the marketplace. When we examine the new product introductions we see that many new product forms of styling mousses and gels are emerging. [3] The styling gel and the mousse markets have been growing globally at a range of 4 - 6% a year with some regions having seen growth up to 10%. Products for the Ethnic - Specific Hair Care Segment, predominantly African- American and multicultural consumers have also been expanded. The directed marketing towards teens, children, and men has been successful with many new innovative products introduced to the marketplace. These products are expected to continue. If we look specifically at regional growth, we observe that in Asia, the men's grooming market is outpacing women's products.

Male grooming products represent about 10% of this market. [4] Gels and mousse products are the most popular. Both flexible and stiff feel are required to meet the needs of this region. High humidity hold is also an important parameter due to the environmental conditions (high heat and high humidity) The leading market drivers for this region are products that provide multi-functional benefits, such as shine, conditioning, smoothing, volume and control, protection, long lasting style, anti-frizz, and moisturizing effects.

Market Leaders:

Based on Euromonitor International 2005 data, [5]the top 12 customers who are leading the market are as follows: L'Oreal is the number 1 market leader with 20.3% market share, P&G is second with 16.1%, Unilever is third with 8.7%, Henkel KgaA is fourth with 7.7%, Mandom Corp is fifth with 5.2%, Shiseido Co. Ltd is sixth with 4.7%, Alberto Culver is seventh with 4.0%, Kao Corp is eight with 3.6%, Private Label Brands is ninth with 3.3%, Colgate Palmolive is tenth with 1.6%, Beiersdorf AG is eleventh at 1.3% , and Sara Lee Corp is twelfth with 0.8% market share. These 12 companies represent $6.09 Billion dollars or 77.4% of the whole styling & fixative market. Looking further at the brands, we observe that the Studio Line Brand is in the top position followed by Pantene Pro-V. Table 2 lists the top 25 brands.

Table 2: The Brand Share Ranking of the top Global brands

Brand Share Ranking	Brand Name	Brand Share Ranking	Brand Name
1	Studio Line- L'Oreal	13	Garnier Grafic- L'Oreal
2	Pantene Pro-V- P&G	14	Clairol Herbal Essences- P&G
3	Taft/Drei Wetter- Henkel KGaA	15	Geraid- Shiseido
4	Garnier Fruitis – L'Oreal	16	Salon Selectives- Unilever
5	Elnett–L'Oreal	17	Nivea Hair Care- Beiersdorf
6	Alberto Culver VO5	18	Shiseido- Shiseido
7	John Freida- Kao Corp	19	Lucido –Mandom
8	Wellaflex- P&G	20	Vivelle- L'Oreal
9	Gatsby- Mandom Corp	21	Gard –Colgate Palmolive
10	Suave – Unilever	22	Brylcream- Alberto Culver
11	Shock Waves –P&G	23	Rave –Unilever
12	Auslese- Shiseido	24	Uno- Shiseido
		25	Sunsilk- Unilever

Ref: Euromonitor International- 2005

Novel Product Forms:

Hair Styling and Fixative products are now addressing style specific hairstyle solutions. Consumers want style versatility. Products are designed to help: straighten, define curls, smooth unruly hair, reduce or eliminate frizz, add volume, be weightless- not build-up, infuse moisture, cover thinning hair, protect from thermal damage, achieve funky hairstyles, achieve multi-functional effects; i.e. hold the hair in place and condition it. The landscape of styling products now includes: tonics, balms, gel of all types, putty/gum type products, mousses, waxes, creams, serums, pomades, elixirs, gel mousses, and lotions and sprays.

Consumer Drivers:

Consumers prefer not to be locked into one style, they want style flexibility. In focus groups, consumers have stated that they want the following attributes for their styling and fixative products: [6]

- Improved Hair Style Hold
- Conditioning
- No Flaking when Brushed or Combed
- Increased Hair Body and Hair Volume
- Good Hair Feel
- Detangling/ Easy Combing

- Good Hair Gloss/Shine
- Stiffness or No Excessive Stiffness
- Easy Application on Wet Hair/ No Clumping
- More flexibility or movement of styled hair
- No Sticky Feel
- Quiek Drying
- Good Stability
- Smoothness
- To Control Frizz
- To Define the Curl

Polymer Requirements for Styling & Fixing:[7]
Typically the polymer provides many of the attributes during styling, as well as the holding and texture characteristics. However the total formulation determines the functionality such as ease of application, distribution of polymer, absence from flaking, shine, longevity of hold, restylability, and removability. This will be discussed further in the program by several of the other speakers today.

In designing a new styling product there are specific polymer attributes that are desired. You may require a product with a particular spreading or distribution characteristic (rheology), in order for it to distribute it through the hair. The degree of tackiness or wetness needs to be optimized so that it is not too tacky or wet. The stiffness, crispness, or slip can also be tailored by choosing a polymer with a specific molecular weight. Typically 500M-1.5MM) MW is a good starting point. Sprayability will also need to be controlled especially for low VOC products where a low MW polymer in the range of (10M-200M) may need to be utilized. Or a host of other effects that will require the addition of other additives for the required feel, shine or hold specified by the consumer's need. As stated previously, the product performance and efficacy is rarely a function of only one active ingredient.

Polymer Properties That Affect Hold

- Molecular Weight
- Glass Transition Temperature, Tg
- Particle Size of Spray
- Spreadability
- Cohesive/Adhesive Balance
- Hydrophobicity
- Ability to Form Strong "Spot Welds"
- Concentration of Active Polymer

There are many polymers to choose from in developing your formulation. The key is to understand the polymer profile characteristics and use conditions to achieve a successful styling and fixative product.

Polymer Offerings Prior to VOC Reformulation:[8]
NONIONICS
(PVP)
(VP/VA Copolymers)
ANIONICS
(Esters of PVM/MA)
(Acetates)
POLYQUATERNIUMS
(Polyquaternium-4, 10, and 11)
CATIONICS
(Cationic VP Copolymers and terpolymers)
AMPHOTERICS
(Acrylate Copolymers)

Polymer Offerings After VOC Reformulation[9]
ANIONICS
(Low MW Esters of PVM/MA)
(Low MW Acetates)
(Maleimide Copolymers)
CATIONICS
(Cationic VP Copolymers and Terpolymers)
AMPHOTERICS
(Low MW Acrylate Copolymers)
POLYESTERS
POLYURETHANES
ACRYLAMIDE COPOLYMERS
POLYVINYLCAPROLACTAMS
POLYQUATERNIUMS
(PQ-4, -10, -11, -16, -28, -39, - 44, -55)
POLYAMIDES

RHEOLOGY MODIFIERS:
New Product forms have changed the whole styling segment category with respect to thickening agents. Besides thickening the product, rheology modifiers create body, prevent dripping, and provide texture to the gel/cream product. Crystal clear, water white gels are still sought but new products are requiring the need for new thickeners to achieve the desired results; i.e. sheer thinning, suspension.

Traditional Thickeners:
Anionic Polycarboxylates
Carbomer
Cellulose Derivatives
Hydroxyethyl Cellulose
Gums
Hydroxypropyl Guar

Expanded Product Range to Address New Rheology Market Needs:
Carbomer
Hydroxyethyl Cellulose
Hydroxypropyl Guar
Acrylates Copolymers
Anionic Liquid Dispersion Polymers (LDP's)
Sodium Polyacrylates and various carriers
Cationic Liquid Dispersion Polymers (LDP's)
Polyquaternium –32 (and) Mineral Oil (and) PPG-1 Trideceth-6
Polyquaternium-37 (and) Mineral Oil (and) PPG-1 Trideceth-6
Polyquaternium-37 (and) Propylene Glycol Dicaprylate/
Dicaprate (and) PPG-1 Trideceth-6

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KEY PROPERTIES OF HAIR FIXATIVES NECESSARY TO MAKE AND EDUCATED CHOICE

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In order to formulate an effective hair styling product it is very important to understand the properties of the ingredients used in the formulation, in particular of the fixative ingredients, that, for the most part are polymers. The following is an outline of some of the properties that should be considered in the choice of the fixative polymer.

Basic technical physical chemical properties of polymers:

1) Chemical nature, ionic, non-ionic, cationic, anionic amphoteric.

2) % solids, or actives. Note that for pre-neutralized polymers the weight of the neutralizer is usually included in the value for % solids.

3) Acid value and suggested neutralization level; relevant only to acid containing monomers (e.g.: acrylic copolymers). The following are the neutralization equations provided by different polymer suppliers, slightly different forms to calculate the amount of neutralizer needed in the formulation:

N = (x y z A) / 100N = quantity of neutralizing agent (**g**)
x = quantity of polymer raw material (**Kg**)
y = acid value for the polymer (**mg / g**)
z = degree of neutralization (no units, **0 < z < 100**)
A = factor for each specific neutralizer (**no units**).
A is the ratio between the molecular weight of the neutralizer and the molecular weight of potassium hydroxide (KOH = 56.1)

(W x A x N x E x S)/1000 = g of neutralizer required
W = Weight of polymer in grams (**g**)
A = Acidity of polymer in (**meq/g**)
N = Percentage of desired neutralization (no units, **0 < N < 100**)
E = Equivalent weight of neutralizer (**meq/g**)
S = Solids content of polymer (no units, **0 < S < 100**)

X = (A B C D)/E 1000
X = neutralizing agent (**g**)
A = acidity of the polymer (**millimoles**)
B = g of polymer used in the formulation (**g**)
C = molecular weight of neutralizing agent (**g/mole**)
D = % of neutralization required by the polymer (no units, **0 < D < 100**)
E = % concentration of the neutralization agent used (no units, **0 < E < 100**)

NOTE: The level of neutralization of the polymer will have an influence on various properties of the polymer, among which water solubility, and ability to be removed from the hair (wash out), compatibility with the formulation medium, particularly in the case of aerosol sprays. In standard hair spray formulations the acidity of the polymer and it's level of neutralization determine the pH of the formulations.

4) Cationic charge, this property id of importance for cationic polymers, depending on the chemistry of the polymer there are preferred methods to report the results, for instance, when the cationic charge is being measured by titration, the result will be reported as equivalent or milliequivalents of titration agent per gram of polymer (or raw material), when the cationic charge is being measured by chemical analysis it may be reported by % nitrogen

5) Certificate of Analysis, it is an excellent idea to always check the CoA of each new raw material that one is thinking of using, this will also alert you to many other information, like, expiration date, level of residual monomers, etc.

Specific physical chemical properties of polymers:

1) Glass transition temperature (Tg): is the temperature (different for each polymer) below which the polymer becomes hard and brittle, like glass. This property is linked to the effect that temperature and humidity may have on the style retention ability of the polymer (or the formulation). In a finished formula or in simply a diluted solution of the polymer, curl retention is a very commonly tested property, this test involves styling the hair, usually in curls, exposing it to controlled humidity and temperature, and monitoring the hold, with time, of the initial size and shape of the curl. The results are often expressed in terms of % curl retention and it is a function of the polymer in combination with all the other ingredients of the formulation.

2) Average molecular weight (Mw), always keep in mind that polymers are never just one exact molecular weight, there is always a distribution. Experimentally measured molecular weight of polymers are dependent on the measurement technique used, and take different names/symbols. In addition, often, for traditional reasons, the molecular weight of polymers is reported in terms of K value. The higher the K value the larger is the average size of the molecules of the polymer. Some definitions related to different molecular weight:

Mn = number average molecular weight; measured by methods that count the number of molecules in the system

Mw = weight average molecular weight, measured by methods in which the size of the molecule determines the magnitude of the response.

Mv = viscosity molecular weight, measured by intrinsic viscosity

Polydispersity = Mw/Mn ; also referred to heterogenaity index.

3) Solubility of the polymer: in water, ethanol or other solvents.

4) Stiffness ability of the polymer, this determines the type of styling action that the polymer will be able to perform. For hair sprays and gels it is usually hold, stiffness or 'crunch', for mousses it can be but usually it takes the form of style, volume and control. There are plenty of variations on basic laboratories test methods for a first assessment of this property.

5) For aerosol spray formulations it is important to know the level of propellant compatibility.

Things to watch out for:

1) Possible impurities of by-products that could be of specific relevance to specific markets, e.g.: Porposition 65 ingredients, CMR (carcinogenic, mutagenic, toxic to reproduction) substances etc.

2) Volatile Organic Compounds (VOC) content. The maximum limit for the content of VOC in different formulations is set by each State separately, or by EPA on a National level. The most affected categories are the ones of aerosols, sprays or mousses, and most recently hair gels. Ethanol and propellants are among the ingredients that are greatly affected by this regulation; note that often ethanol is the solvent for a raw material although the INCI name does not list it.

3) Origin of the raw materials used to produce the polymer: vegetable, synthetic or animal derived.

4) Preservative used in the polymer.

5) Shelf life of the product, age of the sample being used for your development work !

6) Various certifications and commonly used acronyms:
GMO-free (Genetically Modified Organism), Kosher, NF (National Formulary), USP (United States Pharmacopoeia), cGMP (current Good Manufacturing Procedures).

HAIR STYLING OVERVIEW AND FORMULATION APPROACHES

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In recent years, the global hairstyling market has been segmented into numerous product forms and performance choices. Traditional styling formulations, including hairsprays, mousses, and gels, are still considered to be the key applications in a styling product line. This paper will discuss the ingredients and strategies necessary to formulate an effective hairspray, mousse, and gel product.

Hairspray

Hairspray products are typically used to hold a finished hairstyle in place and protect it from falling apart in humid environments. Some hairsprays, particularly non-aerosol products, can be used as working sprays that are applied during the styling process for hair manageability and control. In the United States, most aerosol and non-aerosol hairspray products are 55% VOC compliant formulas due to the CARB and Ozone Transport Commission (OTC) regulations. Typical 55% VOC hairspray formulations, including ingredients and ranges, are listed in Table 1.

The fixative polymer is the key raw material in any styling formulation. The polymer provides the hold, stiffness, and adhesion of hair fibers to fix the style. It also provides high humidity curl retention properties. Resins that have a high molecular weight and modulus (toughness) with some hydrophobic character will typically provide the strongest hold and humidity resistance when effectively delivered from a hairspray system. Examples of common hairspray polymer chemistries for 55% VOC systems include acrylates (Acrylates Copolymer, Octylacrylamide/Acrylates/Butylaminoethyl Methacrylate Copolymer), acetates (VA/Crotonates/Vinyl Neodecanotate Copolymer), esters of PVM/MA, maleimide copolymers (Isobutylene/Ethylmaleimide/Hydroxyethyl Maleimide Copolymer), polyesters (Diglycol/CHDM/Isophathalates/SIP Copolymer), and polyurethanes (Polyurethane-1, Polyurethane-14 (and) AMP-Acrylates Copolymer).

Polymers that contain carboxylic acid groups will need to be neutralized to balance shampoo removability with good humidity resistance. Varying the degree of neutralization can affect several properties of the hairspray system, including solution viscosity, film hardness, film clarity, polymer solubility, tackiness, humidity resistance, removability, and propellant tolerance. These neutralization differences allow the formulator some additional latitude to modify product performance.

When formulating a 55% VOC hairspray, the ethanol solvent and typical propellant(s) (e.g., hydrocarbons, dimethyl ether) cannot exceed 55% of the formulation. Therefore, water (an inexpensive non-VOC solvent) is now becoming a major component of the hairspray system. Higher levels (> 25%) of water in a hairspray formula start to deteriorate product performance, including very slow setting and drying time, high formula surface tension, high formula viscosity, and can corrosion potential. The high surface tension and viscosity impede the polymer droplets from flowing down the hair and forming spot/seam welds, thus providing poor holding power. Solvent and propellant ingredients that are exempt from the VOC rule, including acetone and methyl acetate solvents and Hydrofluorocarbon 152A propellant, may replace a portion or all of the water in the formula to attenuate these performance issues. However, ingredient and packaging compatibility, odor, and cost need to be taken into consideration before using these alternative components.

Additives are becoming more important in enhancing the performance of 55% VOC hairspray systems. They can be used to aid dry combing, reduce flaking by resin plasticization, increase gloss, provide UV protection, improve sprayability, improve flow down the hair, and prevent can corrosion. For example, silicones (dimethicone copolyols) are commonly used to plasticize the resin to reduce flaking and improve combing. Small levels of Cyclopentasiloxane can reduce formula surface tension dramatically, allowing for improved sprayability and flow down the hair. In addition, most high water aerosol hairspray systems now require corrosion inhibitors (e.g., sodium/ammonium benzoate, cyclohexylamine, etc.) to prevent rust in tin-plated steel cans.

Styling Mousse

Mousse products are hair styling tools that provide hold and control, increase body and volume, improve wet and dry combing, improve wet and dry feel, and provide overall manageability during the styling process. The components of mousse formulations can be similar to hairsprays, however they are mainly aqueous and usually contain conditioning polymers and surfactants/emulsifiers to produce a foam. Mousses can be applied from an aerosol foam, aerosol spray foam, or non-aerosol using special pump foamer packaging. The current CARB and OTC regulations for mousses and foams are 6% VOC systems. Typical 6% VOC compliant mousse formulations are listed in Table 1 below.

In a typical aerosol mousse formula, the concentrate and propellant are two separate phases and must be shaken immediately before use. When the mousse is shaken, the propellant is emulsified in the aqueous phase and expands when

it is expelled from the can to produce the foam. The polymer and emulsifier provide the foam stability. Most commercial mousse foams vary in density, strength, and bloom, but must break down quickly when worked into the hair.

The polymers that are typically used in mousse formulas are cationic and provide fixative and conditioning benefits. The cationic resins are substantive to hair, yield good hold, and offer excellent combing and feel in the wet and dry stages. Examples of cationic mousse polymers are Polyquaternium-4, 10, 11, and 16. Nonionic polymers, including PVP and PVP/VA, can be included for enhanced holding power. In addition, acrylic resins (Acrylates Copolymer) can be blended with the cationic polymers to provide improved hold and humidity resistance.

There are a variety of surfactants to choose from when formulating a mousse system, however nonionic emulsifiers will have the widest compatibility range with the other ingredients. Nonionic emulsifier blends of low HLB with high HLB tend to provide the best foam density and foam strength. Hydrocarbon propellants, typically combinations of isobutane and propane, are used in aerosol mousses to force the concentrate out of the can to form a foam. The hydrocarbon or DME propellants and ethanol total cannot exceed more than 6% VOC in a US mousse formula, which can result in thick, rich foams that are difficult to work with. To combat this issue, Hydrofluorocarbon 152A (non-VOC propellant) may be added with the hydrocarbon propellant to reduce foam density and improve foam breakdown.

Styling Gel

Gels also provide hold, control, and a textured look to the hair style. These formulations are higher in viscosity and typically contain a thickener to create body, prevent dripping, and provide texture to the gel product. The current CARB and OTC regulations are 6% VOC for styling gels. A typical gel formulation is listed below in Table 1.

When formulating a gel product, it is important that the polymer and thickening agent are compatible with each other. There are many thickeners to choose from, with each having specific requirements for pH, viscosity build, clarity, and compatibility with salts, polymers, additives, and alcohol. Different thickeners will also provide different rheology and texture. Anionic polycarboxylate thickeners (i.e., Carbomer) are popular choices due to their shear thinning rheology and buttery texture; however, they cannot tolerate high levels of salt and may experience incompatibilities with anionic and cationic ingredients. Therefore, nonionic polymers such as PVP or PVP/VA are typically used as the fixing agents in Carbomer-based gels. Cellulose derivatives (hydroxyethylcellulose), gums (xanthan, hydroxypropyl guar), nonionic synthetics, and acrylic associative thickeners are other examples of thickening agents that can be used in a styling gel formula. These gelling ingredients may need to be neutralized to create the viscosity build.

Although PVP copolymers are still commonly used in gel products, newer styling gel fixatives are being offered to provide improved hold and high humidity curl retention performance while still maintaining acceptable gel clarity. These new polymers are based on polyacrylate, methacrylamide, modified xanthan gum, and polyamide chemistries.

Since gels are primarily aqueous, they require a preservative to prevent microbial growth. Most gel formulations also need an ultraviolet sunscreen to prevent color, clarity, or viscosity degradation when exposed to light. In addition, a chelating agent is recommended to tie up the salts from other ingredients, which will protect the color, fragrance, and gel integrity of the formula.

Table 1: Typical 55% VOC Hairspray, 6% VOC Mousse, and 6% VOC Gel Formulations

Ingredient	Aerosol HS	Non-Aerosol HS	Aero Mousse	N/A Mousse	Gel
Polymer	2-8%	2-12%	0.5-5%	0.5-5%	0.5-5%
Neutralizer (if needed)	calculated based on polymer, level		calculated based on polymer, level		
Additives	0.1-2%	0.1-2%	0.2-2%	0.2-2%	0.1-1%
Ethanol	10-35%	≤ 55%	0-6%	---	0-6%
Water	q.s.	q.s.	q.s.	q.s.	q.s.
Fragrance	0.1-0.5%	0.1-0.5%	0.1-0.5%	0.1-0.5%	0.1-0.5%
Corrosion Inhibitor	0.1-1%	---	---	---	---
Nonionic Surfactant	---	---	0.3-2%	0.3-2%	0.5-1%
Preservative	---	---	0.2-1%	0.2-1%	0.2-1%
Thickener	---	---	---	---	0.25-1%
UV Screen	---	0.1%	---	0.1%	0.1%
Chelating Agent	---	---	---	---	0.1%
Propellant	20-45%	---	6-10%	---	---
Total	100%	100%	100%	100%	100%

PROPERTY AND PERFORMANCE COMPARISON OF HAIR FIXATIVE POLYMERS FOR 55% VOC HAIRSPRAY

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INTRODUCTION

New state VOC (volatile organic compound) regulations went into effect January 1, 2005, in states that adopted the Ozone Transport Commission's model rule for consumer products. Delaware, Maryland, New Jersey, New York, Pennsylvania, and the District of Columbia, as well as California, now limit VOCs in hairspray to 55%. A similar rule in Maine takes effect in May 2005. One approach to reducing VOCs in hairspray is to replace a portion of the ethanol with water. However, if this is done without also changing the hair fixative polymer, the result can be larger spray droplets, greater initial curl droop, and slower drying compared to 80% VOC formulations. Typical polymers used in 80% VOC hairsprays are vinyl polymers such as VA/crotonates/vinyl neodecanoate copolymer, octylacrylamide/acrylates/butylaminoethyl methacrylate copolymer, and esters of PVM/MA copolymer. Suppliers of hair fixative polymers have either modified these existing polymers or developed new ones to accommodate the higher water content of 55% VOC formulations. For example, the viscosity of ethanol/water solutions of vinyl polymers can be reduced by decreasing the molecular weight of the polymer. In the case of the water-dispersible polyester used in an alcohol-free hairspray, the monomer content was adjusted to accommodate 55% ethanol. When selecting a hair fixative polymer for a 55% VOC hairspray, the formulator needs to consider the properties of the polymer and how those properties relate to the desired performance characteristics on the hair.

MATERIALS AND METHODS

The hair fixative polymers listed in Table 1 were evaluated in ethanol/water aerosol concentrates at 7 7 wt% (without propellant) or at a concentration of 5.0 wt% in aerosols with dimethyl ether propellant. The total concentration of VOCs, ethanol (SDA 40-B) plus dimethyl ether, in the aerosol was 55%. The polymers requiring neutralization were neutralized with aminomethyl propanol (AMP). The purpose of this study was to compare polymer properties without the influence of other ingredients. Therefore, no other additives were used in the formulations. The test methods used are as follows.

Polymer Particle Size and Concentration – Particle size and the concentration of polymer particles in dispersion/solution were determined using the Polymer Laboratories PL-PSDA particle size distribution analyzer. The PL-PSDA operates on the principle of packed column hydrodynamic chromatography (HDC), a technique for separating particles based on their size, eluting in order largest to smallest.

Viscosity – The viscosity of the aerosol concentrates was measured using the Brookfield viscometer, model LVDV-I+.

Dry Time – The aerosol formulations were sprayed in a controlled manner on hair tresses and dry time was assessed by feeling the tresses as they dried. Dry time was also determined by dynamic mechanical analysis (DMA). The test developed at Eastman Chemical Company monitors changes in the viscoelastic properties of a polymer film during drying in real time.[1] About 0.1 ml of the polymer solution is transferred to a circular trough (0.2 mm deep) and the surface is smoothed with a straight edge. A T-bar mounted on a dynamic rheometer is lowered into the liquid film. The T-bar oscillates at 10 rad/sec while the film dries, measuring complex viscosity (η^*), viscous modulus (G"), and elastic modulus (G').

Tack – The tackiness of the aerosol formulations sprayed on hair tresses was assessed at the same time as the tactual assessment of dry time. DMA and other methods for determining tack are being evaluated and will be discussed in this presentation.

Gloss – The aerosol formulations were sprayed on lacquer-coated Leneta chart paper to form a continuous film and allowed to dry overnight. The BYK-Gardner micro-TRI-gloss reflectometer was used to measure gloss of the polymer films at a 60° reflection angle.

Curl Retention – Hair tresses of equal weight were washed, dried, hung on a pegboard, and cut to equal length (L). The pegboard is marked with graduations to facilitate length measurements. Tresses were then rewet and curled on ½-in diameter *Teflon* rods. After drying overnight, each curl was carefully removed from its curling rod and hung back on the pegboard. The original curl length (L_0) of each curl was recorded. Each curl was rotated while being sprayed for 4 seconds, then immediately re-hung on the pegboard. After 10 minutes, curl length (L_t) was measured. This length is a measure of the initial curl

droop, calculated as: $[(L - L_t) \times 100]/(L - L_o)$. After measuring initial curl droop for all curls, the pegboard was placed in a high humidity chamber at 22°C and 90% RH. Subsequent curl measurements (L_t) were made after 1, 2, 3, and 5 hours, and the percent curl retention was calculated as given above.

RESULTS AND CONCLUSIONS

The water-dispersible sulfopolyester, Polyester-5, is known to have a measurable particle size in ethanol/water, and therefore is considered to be a dispersion rather than a solution. The particle size results for all five polymers were very similar (mean diameters from 10 to 13 nm), but the concentration of particles was much greater for Polyester-5 compared to the vinyl polymers at the same polymer concentration. This is significant because polymer particles in dispersion release water faster than polymer molecules that are in solution.

The viscosity results are shown in Figure 1. Typically, lower viscosity solutions provide smaller spray droplets. Smaller droplets have a higher surface area to volume ratio, resulting in faster dry times.[2]

The dry times determined by feeling the hair tresses are shown in Figure 2. Dry time determined by DMA shows similar trends. When comparing these results, one needs to consider that the dry time on hair is affected by the sprayed droplet size and the spray pattern (the amount of sprayed solution on the hair), whereas the dry time of the polymer film (DMA method) is independent of these variables.

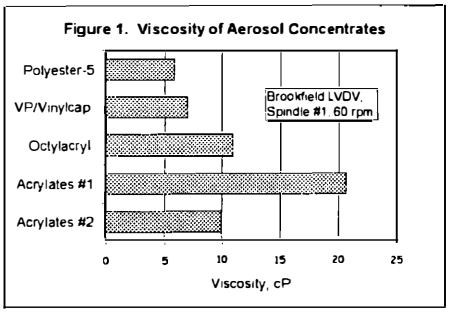
Gloss results show higher gloss for Polyester-5 and VP/Vinylcaprolactam/DMAPA Acrylates compared to the other three polymers.

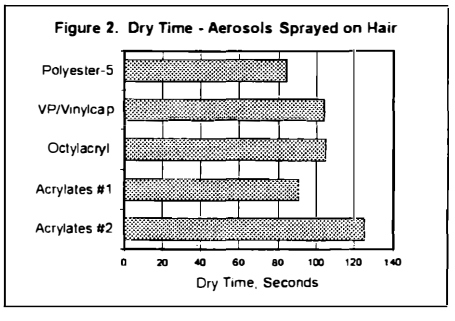
Curl retention results did not show a statistical difference among the five polymers. Five tresses were tested with each polymer formulation. To ascertain significant differences, more tresses would need to be tested per formulation.

Flaking and comb-ability characteristics on hair were also evaluated. No significant problems or differences were noted for flaking and comb-ability among the five polymers.

Table 1. Hair Fixative Polymers for 55% VOC Hairspray

Copolymer INCI Name	Supplier	Supplied as...	Neutralization...
Polyester-5 (formerly Diglycol/ CHDM/Isophthalates/SIP Copoly.)	Eastman	Pellets	Not required
VP/Vinylcaprolactam/ DMAPA Acrylates Copolymer	ISP	38-42% in ethanol	Not required
Octylacrylamide/Acrylates/ Butylaminoethyl Methacrylate Cop.	National Starch	Powder	Neutralized 75% (Acidity: 2.5 meq/g)
Acrylates Copolymer #1	BASF	35-39% in water	Neutralized 100% (Acidity: ~1.0 meq/g)
Acrylates Copolymer #2	National Starch	50% in water	Neutralized 95% (Acidity: 1.5 meq/g)





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EVALUATION OF FIXATIVES

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The previous papers in this section discussed classical and new products, why and how to make them, how to choose and how to use key ingredients, as well as details on what were some of the challenges. This last portion will cover how to characterize the final product both as a product and as to its function on the hair.

Product Attributes (in the jar)

Styling and Fixative products can be characterized by their appearance, application method, container from which they are dispensed, as well as how they perform on the hair in the wet stage and all the stages throughout and after the drying process, like detangling, ease of combing, smoothness, tackiness, stiffness, flexibility, rehydration, response to humidity or dryness, durability, clarity, shine, and removability.

If we start by looking from the consumer's viewpoint, we would look at how the product appears in the package and being dispensed from the package. For a product in a clear package, the first measurement would be the color and opacity or clarity. Whether clear or opaque, next might be the texture or flow of the product as the package is tilted at an angle (or inverted) and possibly poured out or sprayed. At this point the product is also smelled and possibly felt. For aerosols, the first measurements might be the weight of the can, the actual texture and dimensions of the can, the feel or sound as the can is shaken, and then the style of spray followed by the smell and feel on hand, wet through dry. Some of these characteristics may be the foundation for specifications to ensure the product meets the profile and maintains consistency from batch to batch.

Quite often, we measure the texture of a product by viscosity. During product development, 2 or 3 shear rates may be employed to take a 'picture' of the response of the product to different situations. For example, to evaluate a product just sitting on the shelf, being poured, or suspending something dense or light, low shear may be used, perhaps 0.5 – 12 rpm. For those products needing to be spread or sprayed, higher shear is used, 30 – 100 rpm. Choosing the wrong shear rate can provide false information. For example, using an inappropriately high shear rate (100 rpm) on a product that shear thins at medium or high shear rate can make a product appear to be thin with low centipoise (cps) readings. However, upon a visual or tactile investigation, the product could be very thick when at rest. The flip side is true when evaluating a product that needs to be thin during application of high shear, like a styling gel in a tube to be spread through the hair, a thickened spray gel, a pump spray, or canned aerosol spray. They need to be evaluated at higher rpm to help predict or quantify how they will respond to the high shear. Of course, testing the final applicator in the lab is critical to choosing the delivery orifice etc. prior to setting specs. Controlling the temperature and choosing the spindle or T-bar and container size can be just as important as setting the rpm for measuring the attribute on which you are focusing.

If you consider lotions (water based styling polymer solutions with no thickeners), glazes or serums (water based systems with low levels of thickeners or just soft textures), thick gels (shear thinning clear or opaque), pump and aerosol concentrates, the Brookfield viscometer is still the standard, although others instruments are employed. When reporting the cps, it is often helpful to include the Factor along with the Spindle, speed/rpm, temperature, and timing so it is possible to determine if the cps differences are significant. When it comes to the pastes, muds, glues, sticks, water-waxes, and pomades, evaluations have to move to a Penetrometer type measurement or other bending or smashing methods.

For clear systems, both NTU (Nephelometric Turbidity Unit) and %T (percent transmission) are quite often used to quantify the degree of clarity. Low NTU (low haziness) and high %T are required for clear systems. For colored systems, visual comparisons to color chips or vials of colored solutions are often employed. However, absorption or reflection spectrophotometry (sometimes using the L a b system) is used for products as well as packaging. Regardless of color or clarity, odor, before and after the fragrance is added, is important to note, as well as during stability tests.

Products we make don't get used the same day they are produced, so microbial or preservative challenges must be evaluated as well as stability at RT, 4^0C, Freeze/Thaw, and elevated temperatures (accelerated aging) to ensure that the consumer can use the product and achieve the performance expected. What needs to be

measured over a 1-3 months (or 1-2 yrs) timeframe? Any attribute that is critical to make this product perform according to the profile may be tested. Quite often, stability tests are confined to viscosity, pH, odor, appearance, but could add sprayability, texture or spreadability, suspended air or particles, dispersion particle size, microscopic analysis, particle size of spray droplets, and a host of other key attributes designed into the product for performance or consumer utility.

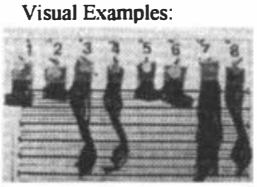
Performance Attributes (on hair)

Now that the product is made and characterized while in the container, it needs to be tested for performance as it leaves the container and is used on hair. Sprays have some particular requirements, like pressure (at 21°C) particle size analysis upon spraying (Malvern), grams per second (g/s) or grams per pump –delivery rate of being sprayed (how much product leaves the can and may hit the hair), propellant tolerance, cone diameter (what pattern the sprayed product covers), wetness, beading on the hair, and forcefulness of the spray as well as corrosion in the can or clogging upon spraying and drying. All these attributes affect the way the product is delivered as well as functions on the hair. Gels and sprays alike can be evaluated for clarity upon dry-down, stickiness and stiffness during drying and after drying at low and high relative humidity (%RH), shine, and smoothness of the film. Mousses have similar requirements plus the foam appearance, texture, spreading, foam density (g/mL) and stability in glass bottles as well as final cans with various linings.

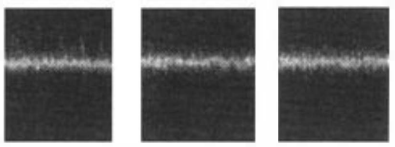
Very important to consumers is the affect on human hair. Hair Characteristics (HC), High Humidity Curl Retention (HHCR), bending studies at low and high %RH, tack and dry time, smoothness (Diastron Combing), tangles, and shine are all tests that relate to consumer and style demands. Actual curling, flattening, blow drying, brushing, and combing treatments are tested on controlled human hair swatches, mannequins, take home tests, and salon ½ head and whole head tests in order to compare and measure multiple consumer required characteristics: shine, stiffness, durability/flexibility (DHSA), curl snap, flaking on the hair and comb, curl memory/manageability, static, feel going on, drying and dried, then after 8 hrs, 24hrs, etc. Can it be reactivated, reapplied, and removed without damaging the hair?

These are just a sampling of typical methods and tests that can be employed to determine if the prototypes meet the objectives of the profile, pass stability, have completed scale up, or confirm differences from the competition or benchmark. Your projects will have its own selection of key attributes that you will want to measure.

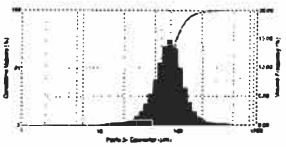
Visual Examples:



High Humidity Curl Retention



Digital Image Analysis: Shine



Spray Particle Size: Malvern Analysis

PROTECTING THE SKIN AGAINST OZONE

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Introduction

Ozone (O_3) can be considered to be a transitory mixture of oxygen (O_2) and singlet oxygen ($^1O_2$) and is an extremely powerful, non-radical oxidizing agent.[1] It has been well documented that ozone will readily kill microorganisms and it is frequently used as a sterilizing agent for this purpose. Unfortunately, ozone is also the most significant contaminate in urban air pollution and when local governments speak of pollution alerts, they are typically talking about unhealthy levels of ozone.[2] Typical levels of ozone that ar recorded in urban environments can range between 0.2 and 1.2 ppm with the higher levels being considere a respiratory threat to people with impaired breathing.[2]

Ozone ravages the human respiratory system and also is now identified as a important source of oxidative stress and reactive oxygen species in the skin.[3-5] In particular, ozone has been identified as a source of depletion of cutaneous vitamins[3] and lipids[4-5]. In addition, it is well established that ozone can damage proteins and nucleic acids although such studies on skin have been sparse.[6] More recently, it was suggested that atmospheric ozone may adversely effect the immune response in frogs, in particular the ability of pulmonary macrophages to function correctly, and that this effect may partly account for the decline in these species.[7] It has also been suggested that certain cells in the human body may actually produce ozone as a mechanism of bacterial control although the results of this initial study have been questioned.[8-9] Interestingly, to date, it appears that there has only been a single *in vivo* study conducted on human skin exposed to ozone[10].

It is well known that growing yeast, in particular *Saccharomyces cerevesiae* or Bakers yeast, responds to threats in a very similar fashion as human skin cells, creating protective agents that can hold powerful oxidizing forces at bay.[11] Because of this, bioactive yeast ferment extracts have been sold in therapeutic, cosmetic and skin care products for years.[12] It has been shown that yeast will respond to oxidative stress and examination of the effects of ozone on yeast have been reported.[13] This paper will examine the *in vitro* effect of ozone on some key cutaneous targets not previously examined including DNA damage, and cholesterol and melanin degradation and will demonstrate the ability that a yeast lysate made from ozone-stressed yeast can offer a protective barrier against topical ozone assault.

Methods

Ozone-Stressed Yeast Extract- The stressed yeast extract used in this study was made by growing *Saccharomyces cerevesiae* under standard conditions and supplementing a controlled amount of ozone into the oxygen feed during the active growth stage. The lysate was isolated by fracturing the yeast and purifying the contents to remove water-insoluble residues.

In Vito Tissue Models- A customized chamber was designed that allowed the growth of MatTek® tissue models including Epiderm®, and Melanoderm® under a controlled atmosphere of ozone co-mixed with the oxygen provided to the tissue. Test materials were applied directly to the surface of the tissue. Analysis of 8-oxoguanine DNA damage was conducted using a competitive ELISA-based analysis. Analysis of tissue cholesterol was conducted using Thin Layer Chromatography and laser densitometer to quantitatively determine content. Melanin analysis was conducted using standard UV spectrophotometric analysis using melanin standards.

Results

Graph 1 shows the DNA damage test results from exposure of Epiderm full thickness tissue to 10 ppm of ozone for 1 hour. Graph 2 shows the results of cholesterol analysis after exposure to 10 ppm of ozone for 2 hours. Graph 3 shows the results of melanin concentrations as a result of 10 ppm ozone exposure for 1 hour.

Graph 1. 8-Oxoguanine levels in Epiderm tissue as a result of exposure of the tissue to 10ppm of ozone for 1 hour. PBS is a negative control and Trolox is a positive control.

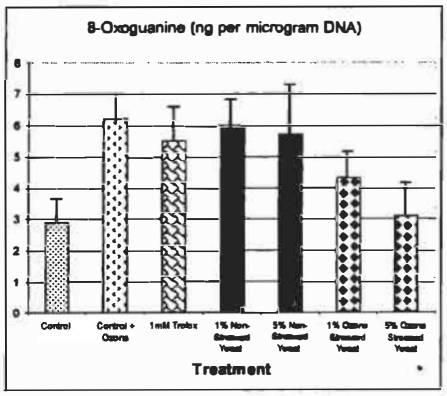
Graph 2. Cholesterol levels in Epiderm tissue as a result of exposure of the tissue to 10ppm of ozone for 2 hours. PBS is a negative control.

Graph 3. Melanin levels in Melanoderm tissue as a result of exposure of the tissue to 10ppm of ozone for 1 hour. PBS is a negative control and Trolox is a positive control. Light bars are 1 hour after exposure and dark bars are 24 hours after exposure.

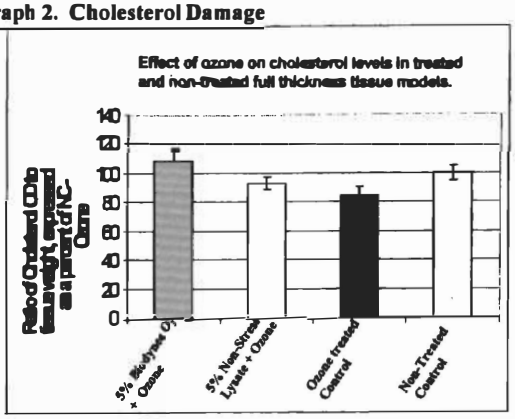
Conclusions

The topical application of the ozone stressed yeast extract protects the Epiderm tissue against oxidative damage to both DNA and cholesterol, but not to degradation of melanin in the Melanoderm model.

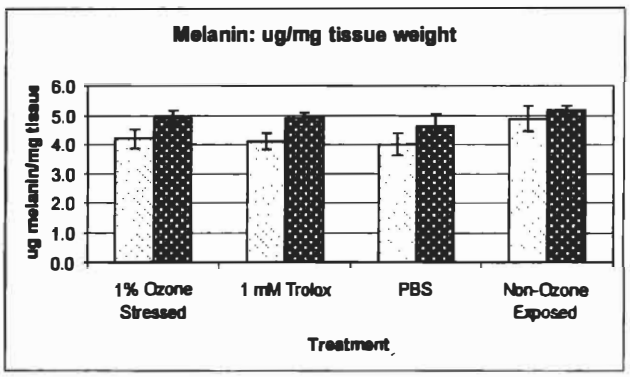
Graph 1. DNA Damage



Graph 2. Cholesterol Damage



Graph 3. Melanin Damage



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LAMELLAR DELIVERY SYSTEM FOR TARGETED DELIVERY INTO THE SKIN

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Abstract

The diversity of applications in cosmetics creates the need to target different compounds into sub-tissues of the skin. While, for example, moisturizers need to be retained in the upper layer of the skin, anti-aging active compounds need to reach the epidermis-dermis junction. An appropriate delivery system will enable better targeting of a compound to its site of action in the skin, while reducing the concentration needed to achieve an effect, and thereby elevating its safety.

We present a novel microscopic lamellar delivery system (MLDS) that can be tailored to target active compounds to sub-tissues in the skin.

This patent pending technology blends specific lipophilic compounds that when combined with water in certain ratios create unique microscopic structures. The composition and processing determines the physical properties of the delivery systems created.

Transmission electron microscopy resulted in the observation of three major structures. It is hypothesized that each of those structures will interact differently with the skin, and allow penetration to the different layers. Skin permeation studies will be conducted to substantiate the hypotheses.

The importance of targeted delivery

Mammalian skin is an excellent barrier for the penetration of compounds. Its upper layer, the stratum corneum, has a special organization where the dead corneocytes are cemented by intercellular lipid lamellae that are covalently linked to the cell membrane (1). The intercellular lipid lamellae are composed of cholesterol, cholesterol derivatives, free fatty acids and ceramides, with the latest being the main constituent (around 50%). Those lamellae are derived from granules, which secrete their content to the intercellular spaces of the upper layer. They include lipids, hydrolytic enzymes and several other proteins (2).

The skin is a multi-layered organ, in which every layer displays diverse composition and properties, and is responsible for different functions. Therefore, when treating the skin, it is crucial to assure that the active ingredient in the applied formulation will reach its target. While sunscreen formulations, for example, should be designed to protect the upper layer of the skin, formulations that include skin-lightening actives should reach the living epidermis. Tailoring an appropriate delivery system can not only enable targeting the active compound to its site of action, but also control its time of residence and release, hence, improve its bioavailability and broaden the therapeutic index for safety.

Lamellar delivery systems - short overview

Studies have demonstrated that the stratum corneum lipid organization can be imitated with model lipid mixtures based on isolated stratum corneum lipids (3). Other systems that were composed of different combinations of cholesterol, fatty acids and ceramides were used as models to test for skin permeation of compounds (4).

Close shaped lamellar systems that are being used as delivery systems for the skin are vesicular systems. Throughout the past two decades scientists have designed a variety of vesicular systems that are meant to embody the active compound in its compartments and carry it (5,6). It was found that while classic multi-lamellar phospholipid made liposomes tend to create a reservoir of the entrapped compound in the upper layer of the skin, special flexible vesicles were demonstrating penetration to deeper layers and even allow for transdermal delivery (7). The common concept behind the design of these systems is to compose an intact relatively stable system that will transmit the active to or through the skin while reversibly changing the stratum corneum barrier properties, and preferably protecting the active compound.

Microscopic lamellar delivery system- hypothesis and rationale

MLDS technology suggests a novel approach, applicable mainly in the area of cosmetic and personal care, but also with potential applications in trandermal delivery. As already mentioned, the skin is considered to be multi-compartment organ in which every compartment (i.e., sub-tissue) is a target for a variety of actives used to treat the skin. The MLDS system, as oppose to previously describe lamellar systems, is used as a "key" to open pathways in the stratum cornem, and not necessarily as a carrier. This is a dynamic system. Negative staining transmission electron microscopy observation showed that the system is composed of three major lamellar structures that are most likely to exist in equilibrium. By changing the percentage of components in the system, and its way of preparation, one can create a situation in which one structure will dominate on the other two.

The three structures are: intact vesicles, with an average size of 100nm, ruptured vesicles (with loose unilammelar membrane) and lamellar sheets (see figure number 1). Based on these structures properties and the understanding of their possible interactions with the skin, it is hypothesized that each system will allow penetration to different sites in the skin. When changing its composition and creating a system where one structure will dominate the others, not only will it affect the skin intercellular lipids differently, but it will change the active's dissolution properties, its partitioning and diffusivity into the stratum corneum and hence its penetration profile.

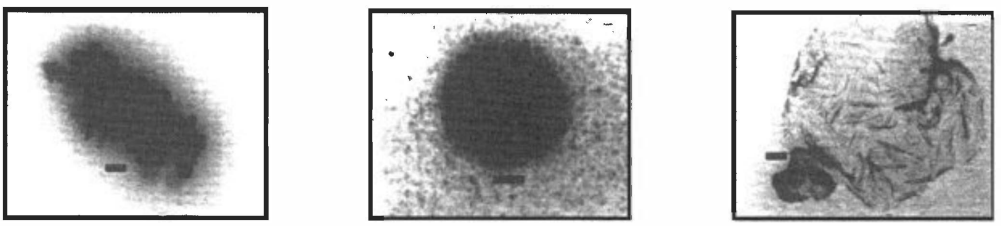

Figure number 1: Three structures were found in the MLDS system, existing in equilibrium (from left to right): intact vesicles, ruptured vesicles and lamellar sheets

Future studies

Differential scanning calorimetry (DSC) will be conducted to follow thermodynamic changes in the system. While moving from organized intact vesicles towards lamellar sheets, alterations in the transition temperature are expected.

In vitro skin penetration studies with marked delivery system will allow to study the correlation between the system structure and its interaction with the skin.

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SILK-ELASTIN PROTEIN POLYMER: A MULTIFUNCTIONAL ACTIVE INGREDIENT FOR SKIN CARE

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Abstract

Biotechnology based products for personal care applications are appearing in the market place. These products fall into several categories: (i) peptides or small proteins, (ii) unique proteins and (iii) catalytic proteins (enzymes). Genetic and protein engineering enables us to engineer and produce protein polymers with multiple domains that can be selected for their unique properties and assemble them together to impart desired functions.

This podium presentation will illustrate a new concept of hybrid proteins designed to deliver multifunctional activity in personal care formulations. We will describe studies (*in vivo* and *in vitro* models) of a novel silk-elastin protein biopolymer as a new functional active ingredient for skin care that has properties of both silk and elastin. This unique protein not only delivers soluble silk and elastin, but also many functional attributes, which are useful in skin care applications.

Introduction

Advances in genomic research offer a unique opportunity to design proteins with specific, targeted properties, that are important for providing specific benefits and that can then be produced consistenetly via fermentation. Additionally, multiple peptide motifs can be engineered to provide protein-based multifunctional biomaterials. Interest in repeat sequence protein-based polymers (RSPPs) has grown because this new class of biomaterials simulates naturally occurring ones, and can be modified for desired function for applications in personal care. Protein engineering offers the ability to screen for desired properties utilizing the tremendous potential diversity of amino acid combinations and fermentation allows for large-scale manufacturing. Using recombinant methods, one can precisely control and incorporate the molecular weight, size, stereochemistry, and functional distribution of active domains in the biopolymer to create self-assembling composite functional biopolymers for personal care applications. Using the twenty natural amino acids, one can create RSPPs designed for a specific function(s). Subsequent chemical/biological modifications of amino acid side chains with a variety of functional groups further offers a means for incorporating specificity and variety in function. Multifunctional bioengineered personal care peptide ingredients can be created that will serve as a new paradigm for a protein-based personal care delivery platform.

RSPPs produced though molecular biological design and fermentation, targeted to incorporate the needed characteristics for personal care applications, have been investigated (1). Representative examples of natural small peptide-based RSPPs and their block copolymers (repeated amino acid sequences,), include elastin, silk fibroin (GAGAGS), byssus (GPGGG), flagelliform silk (GPGGx), dragline silk, collagen, and keratin. The relative stability of these families of structural proteins in combination with their biocompatibility, and unique mechanical properties, provide the foundation on which one may exploit naturally derived RSPPs for wide-ranging personal care applications.

RSPPs are similar to a chemically polymerized block of copolymers but do not have any heterogeneity. They are unique, defined, monodispersed, and have molecular weights that generally range between 30 kD and 250 kD. For example, in a RSPP named SELP47K (silk elastin like protein; Unit block structure: Figure 1), individual units are composed of silk fibroin (S = GAGAGS), and elastin (E = GVGVP), and a lysine modified elastin peptide, K (GKGVP).

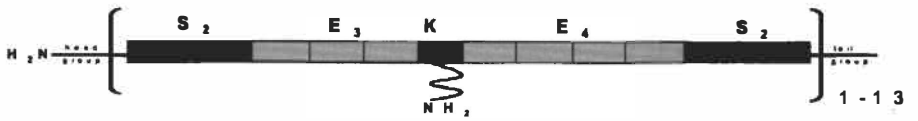

Figure 1: Schematic block representation of the protein polymer SELP47K

SELP47K consists of four silk repeat peptides, seven elastin repeat peptides, and one lysine modified elastin repeat peptide. The latter peptide introduces a potential cross-linking functionality and opportunity for additional chemical modification. This modification also increases the water solubility of the polymer and imparts cationic character for improved substantivity.

Results from *in vitro* studies indicate that SELP47K offers unique properties such as self-assembling nanofiber formation, mechanical strength, and hydrogel matrix development [2]. Additionally SELP47K stimulates human dermal fibroblast cells in-vitro to produce elastin in a dose dependent manner. (Figure 2) [3]. We hypothesize that self-assembling nanofibrillar networks of SELP47K relay a signal to fibroblasts to improve their elastin production.

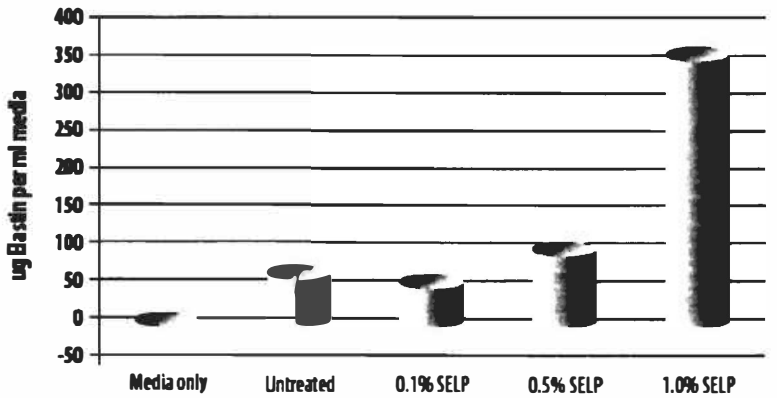

Figure 2: In-vitro assay of elastin production using dermal fibroblast cells

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SKIN LIGHTENING AND ANTI-AGING INGREDIENTS - HOW ARE THEY INTERLINKED?

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The pigmentation of the skin, due to synthesis and dispersion of melanin in the epidermis, is of great cosmetic and societal significance. It is also the key physiological defense against sun-induced damage, such as sunburn, photoaging and photocarcinogenesis. This presentation focuses on polyphenolics of natural origin having both skin lightening and anti-aging effects and their mechanistic interlink which provides these two desired skin benefits.

Photoaging & Melanogenesis: The unifying pathogenic agents responsible for photo-damage are UV-generated Reactive Oxygen Species (ROS) that deplete and damage the enzymatic and non-enzymatic antioxidant defense systems of the skin, and the release of matrix metalloproteases (MMPs) such as MMP-1 and MMP-3, that damage the extracellular matrix proteins[1]. The drastic long-term effects of UV on the skin include photoaging, characterized histologically by solar elastosis due to degradation of collagen and the accumulation of abnormal elastin in the dermis, and skin cancers.

ROS, especially, superoxide anion has been shown to activate tyrosinase thereby increasing pigmentation. Quenching of superoxide anion can also lighten skin[2]. Melanin forms through a series of oxidative reactions involving tyrosine in the presence of tyrosinase. It has been shown that cells, such as, eosinophiles, neutrofiles and mast cells are capable of synthesizing melanin without the presence of tyrosinase. Okun et al were also able to show a correlation between peroxidase-H_2O_2 activity and its ability to oxidize tyrosine or DOPA to melanin[3]. There is no doubt that DOPA can be a good oxidizable substrate for peroxidases and it has recently been reconfirmed that the peroxidase-H_2O_2 system alone is capable of converting DOPA and dopamine to melanin. The ability of the peroxidase-H_2O_2 system to promote the oxidative polymerization of 5, 6-dihydroxyindole and 5, 6-dihydroxyindole-2-carboxylic acid to melanin pigments has also been demonstrated. Recently, the role of NO and Fe^{2+}/H_2O_2-induced melanogenesis have been demonstrated[4]. The later reaction is known as Fenton reaction, which is responsible for generating ROS and thus causing photo-damage to skin.

Cause and consequences of UV-Induced skin damage and their interlinking to melanogenesis can be schematically represented in Figure 1.

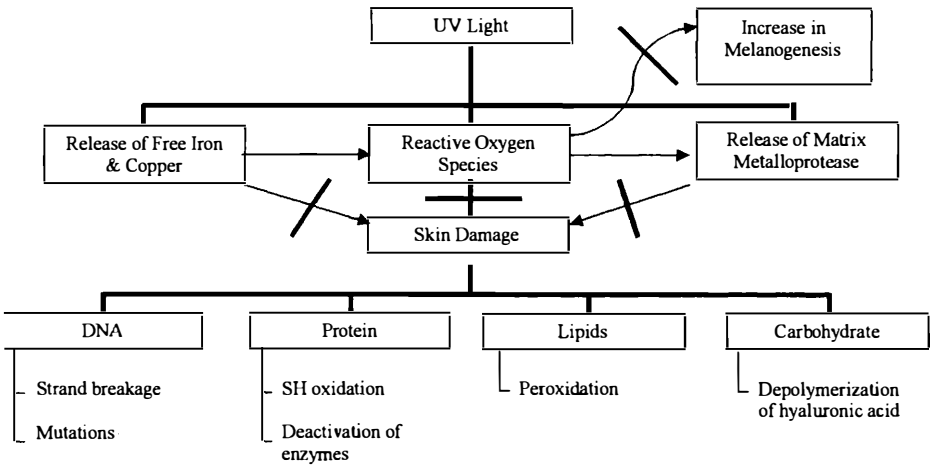

Figure 1: Cause & Consequences of UV-Induced Skin Damage and their Interlinking to Melanogenesis

Eumelanin & Pheomelanin: Eumelanin is best known for its photoprotective role in the skin. Photoprotection is afforded by the ability of melanin to serve as a physical barrier that scatters incident UV light, and as a filter that reduces the penetration of UV light through the epidermis. An important property of eumelanin is its ability to scavenge free radicals, and to function as a superoxide dismutase that reduces reactive oxygen to hydrogen peroxide. Therefore, decrease in eumelanin increases the risk of skin damage from oxidative stress. This can be counter acted by selecting an anti-aging ingredient(s) or a skin lightening ingredient with built-in broad-spectrum antioxidant functionality.

Pheomelanin, which is yellow-reddish in color, differs from eumelanin that the building blocks are derived from cysteinyl-DOPA. Epidemiological data indicate individuals with fair skin are more susceptible to skin cancers than their darker counterparts as pheomelanin exhibits a greater phototoxicity than eumelanin. In contrast to eumelanin, pheomelanin is photolabile and potentially phototoxic. This detrimental effect can also be prevented by supplementing with broad-spectrum antioxidant(s).

Skin Lightening & Anti-Aging Ingredients: A wide range of polyphenolics are known to have skin lightening and also anti-aging properties. Melanin inhibitory activities of natural polyphenolics, such as anthaquinones, arylbenzofurans, chalcones, coumarins, flavonoids, stilbenes, low-molecular weight tannins,etc., have been reported. Skin lightening agents can inhibit melanin biosynthesis by blocking various points of the pathways and are thus useful in lightening human skin. Skin lightening agents can also be used to treat local hyperpigmentation or spots that are caused by local increase in melanin synthesis or uneven distribution.

In order to show the interlink between the skin lightening and anti-aging ingredients, we have chosen two standardized plant extracts belonging to two types of polyphenolics, namely, flavonoids of *Glycyrrhiza glabra* (Licorice, Glabridin as the active) and low molecular-weight tannins (<1,000) of *Phyllanthus emblica* (Emblica). Work done in our laboratory and elsewhere has shown that both products are very effective skin lightening and anti-aging agents due to their broad-spectrum antioxidant and chelating activities. Licorice is also an excellent tyrosinase inhibitor.

Inhibitory concentration (IC_{50}) of peroxidase/H_2O_2 and Fe^{2+}/H_2O_2 induced conversion of DOPA to DOPAchrome for Licorice and Emblica and their antioxidant activity profiles are summarized in Table 1. Clinical trials have shown their effectiveness both as skin lightening and anti-aging ingredients.

Table 1: Comparative In-Vitro Skin Lightening and Antioxidant Activity Profile

	Skin Lightening Efficiency $IC_{50\%}$ (µg/ml)				Antioxidant Activity $IC_{50\%}$ (µg/ml)	
	Peroxidase / L-DOPA	Tyrosinase / L-DOPA	Tyrosinase / tyrosine	Fe^{2+}/H_2O_2	Singlet oxygen	Superoxide anion radical
Emblica	500	140	70	690	60	12
Licorice	430	10	<10	455	20	43
Kojic acid	220	35	<10	150	Pro-oxidant	Pro-oxidant
Hydroquinone	610	230	<10	Melanin ↑	107	400
Ascorbic acid	88	63	30	105	Pro-oxidant	27
MAP	No activity	No activity	No activity	Melanin ↑	500	No activity

Conclusion: What is really needed to create true skin lightening products with anti-aging benefits is to select one or more natural polyphenolics. Tyrosinase inhibitory activity is not a prerequisite to have skin lightening effect. Inhibition of alternate oxidative pathways, namely, peroxidase/H_2O_2 and Fe^{2+}/H_2O_2 can also provide desired skin lightening activity. Skin lightening ingredients can also work by other mechanisms. For anti-aging activity, we do need ingredients with a quencher for ROS, a chelator for iron and copper and an inhibitor for matrix metalloprotease. Oxidative pathways in melanogenesis and the UV-induced oxidative stress are the interlinkage between skin lightening and anti-aging ingredients.

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CORRELATING SENSORY PERCEPTION TO THE RHEOLOGICAL PARAMETERS OF EMULSIONS: A PREDICTIVE MODEL FOR FUTURE PRODUCT DEVELOPMENT?

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Introduction

Although the rheological characteristics and consumer skinfeel properties of emulsions have both been studied extensively over the last few decades, very few published studies have been devoted to finding a correlation between the two. Brummer and Godersky [1] broke down the skin feeling aspects into two groups: "Primary" (initial application) and "Secondary" (final rub-in). They correlated a product's "primary" skin feeling with two rheological measurements: maximum (zero-shear) viscosity, and yield value. "Secondary" skin feeling was correlated with the product's viscosity at approximately 5000 sec^{-1}. Products that fell within the limits for each of these parameters were deemed pleasant-feeling, while those that fell outside those limits were deemed unsatisfactory. Lee et al [2] found a correlation between the G'/G" crossover point stress (what they termed a "critical shear stress") and a skin feeling index score. Wortel et al [3] used multivariate methods to determine that cohesiveness scores could be correlated to the combination of yield value and dynamic viscosity.

For the present study, we are attempting to find if there are any other rheological parameters that could be correlated with skinfeel, and whether more than one are working in concert to generate a particular feeling on the skin.

Materials and Methods

Rheological Analysis: Six different o/w emulsions (with widely differing skinfeel properties) were analyzed at 25°C with an AR-1000 rheometer (T.A. Instruments), using a 40 mm serrated parallel plate, a 1000 micron gap, and a solvent trap (to prevent edge drying).

Three tests were performed on each sample: Stress Sweep, Creep, and Flow. During stress sweeps, the samples were exposed to increased oscillatory stresses, ranging from 0.1 to 200 Pa (log mode, 20 points per decade, 1 Hz), until the samples yielded. For creep tests, a stress value from within the linear viscoelastic region (LVR, obtained from the stress sweep) was chosen, and applied to the sample for 30 minutes, then removed, with the sample being allowed to recover for 90 minutes. For flow tests, a two-step method was used. A steady-state flow test was employed from 10-100 Pa, while a continuous ramp was employed from 1-1000 scc^{-1}. These 2 data sets were then merged to produce one continuous flow curve.

Sensory Panel Analysis: All emulsion samples were submitted to a Skinfeel Spectrum™ Descriptive Analysis panel, which uses physical intensity references for each product attribute, strict protocols for manipulation, and precisely defined terms to discriminate and describe the sensory properties of a given sample [4]. Scores are given for characteristics such as spreadability, firmness, and cohesiveness, with an intensity scale of 0-100 (100 = Very High). Data is then analyzed for significance.

Data Analysis: Rheological and sensory panel data were analyzed (utilizing univariate and multivariate techniques) for correlation and regression with Minitab statistical software.

Results and Discussion

In all, 44 rheological data points were obtained for each emulsion, and these were run against 21 sensory data points. Using regression analysis, we were able to uncover a number of interesting correlations (See Table 1). Cohesiveness, for example, correlates negatively with gel strength at the G' = G" crossover point (Table 2, Figure 1). Three of the five correlations involve multiple variables; each of these three incorporate data derived from the Creep analysis (relaxation time, equilibrium compliance, and minimum [recovery] strain).

Conclusions

Through this work, we were able to uncover a number of correlations heretofore unreported in the literature, most involving data derived from the Creep analysis. Further work may reveal even more correlations. Ultimately, the information derived should lead to a predictive model for future product development.

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Table 1

Sensory Characteristic	Correlated With
Firmness	Zero-shear Viscosity (from Creep test)
Spreadability	Relaxation Time (Creep) + Crossover Stress (Stress Sweep)
Cohesiveness	Gel Strength (G'=G", Stress Sweep)
Cohesiveness	Equilibrium Compliance (Creep) + Viscosity @ 2 inverse sec (Flow)
Integrity of Shape	Relaxation Time (Creep) + Minimum Strain (Creep)

Table 2

Sample	Crossover Point G'=G" (Pa)	Cohesiveness-Pickup
A	53	25.9
B	28	26.7
C	136	15
D	55	31.8
E	160	8.1
F	90	9.6

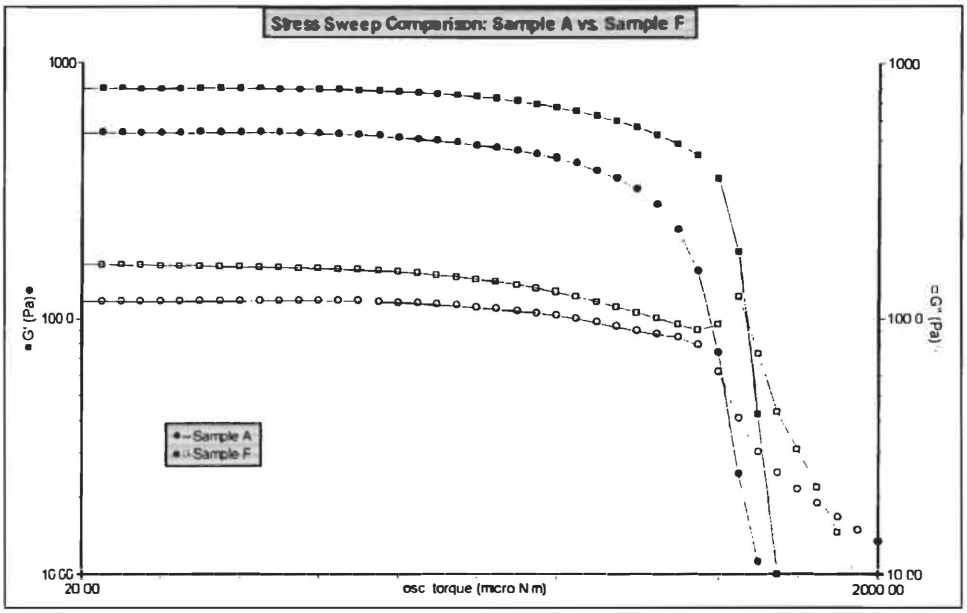

Figure 1. Stress sweeps of A and F. Note the locations where G'/G" crossover occurs.

STRATEGIES OF PRESERVATION

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Introduction

A preservative is a chemical agent that will either kill or prevents the growth of microorganisms that are introduced during consumer usage in those product formulations that are susceptible to microbiological contamination in order to prevent adverse risks for both the user and the formulation itself To prevent microbial contamination of product formulations during consumer usage, there are certain strategies of preservation that need to be evaluated before a preservative system is incorporated into a product formulation.

Regulatory Status of Preservatives

Before selecting a preservative, it is important to determine whether a preservative can be used in a formulation that could be sold in a particular country or geographic region. The problem is that not all preservatives can be used in all countries. For example, the European Communities Cosmetic Directive and the Japanese Ministry of Health and Welfare have always regulated the use of preservatives in cosmetic products by having positive lists. However, preservatives are not regulated in the United States other than that the Food and Drug Administration (FDA) requires that manufacturers have substantiated their safe use in cosmetic products. However, the FDA does have the ability to restrict or prohibit the use of a preservative due to safety reasons. In the absence of an official United States preservative list, the Cosmetic Ingredient Review (CIR) reviews and assesses the safety of cosmetic ingredients such as preservatives. Due to the discrepancies in whether a preservative can or cannot be used in a particular geographic region or country, a cosmetic company has to be diligent when formulating products with preservatives.

Formulation Factors

The following formulation factors can have an affect on preservative activity: water activity of a product formulation, pH of a product formulation and solubility of preservatives. Microorganisms need a source of water for cellular metabolism and growth. By reducing the amount of water in a formulation, microorganisms will be affected by having a longer generation time or reduce metabolic activity. Water activity is different from water content of a formulation. Water activity is a measurement that is used to determine the amount of unbounded water in a system that is necessary to sustain the metabolism and growth of microorganisms. Water activity can be used as a risk assessment tool to identify those product formulations that are susceptible to microbial contamination during consumer usage. For example, nail enamels, lipsticks and powders are examples of product formulations that have low water activity readings and are less susceptible to microbial contamination because they are anhydrous. Creams and lotions are examples of product formulations that have high water activity readings and are susceptible to microbial contamination because they usually contain high concentrations of water. Besides using preservatives, a slight reduction in the water activity of a formulation can be helpful in preventing the growth of microorganisms by increasing the lag phase of the microbial growth cycle. Examples of ingredients that can be used to lower the water activity level of a product formulation by absorbing water are as follows: glycerol, butylene glycol, propylene glycol, dextrin, xanthan gum, sodium chloride, and ethanol. By using a high sugar concentration (e.g. glucose, sucrose, sorbitol), it can also decrease the water activity of a formulation, but also cause an increase in the osmotic pressure.

Most microorganisms are able to proliferate at a pH between 4.0 and 10.0. However, there are some microbial species that can grow below and above this pH range. For bacteria, the optimum pH for growth is between 5.5 and 8.5. For yeast and mold, the optimum pH for growth is between 4.0 and 6.0. If a product formulation has a pH below 4.0 or greater than 10.0, there may be no need to include a preservative because the function of many microbial enzymes is pH dependent. In addition, the pH of a formulation may contribute in making injured cells more susceptible to the antimicrobial activity of preservatives. Besides affecting the growth of microorganisms, the pH of a formulation can affect the antimicrobial activity or the chemical stability of many preservatives.

The solubility of preservatives is an important factor to consider in preserving product formulations because microorganisms will proliferate only in the aqueous phase of a formulation. Some preservatives are water-soluble and can be added directly to the aqueous phase of a formulation. While there are other preservatives that have limited water solubility, a water miscible solvent has to be used to dissolve them or use heat in order to incorporate them into the aqueous phase. In addition, there are preservatives that are more soluble in oil than in water and will partition themselves between the aqueous and oil phases of an emulsion. It is important to control the partition coefficient of these preservatives that exhibit both oil and water solubility to have an adequately preserved product formulation.

Microbiological Activity of Preservatives

When using preservatives, it is important to known the antimicrobial spectrum and recommended use concentration of a preservative or preservative system. There are very few preservatives that have a very broad spectrum of antimicrobial activity against both bacteria and fungi. Most preservatives have either good antimicrobial activity against bacteria or fungi. It is important to incorporate a combination of preservatives by using 2 or more types of preservatives or a preservative blend into a product formulation that will provide antimicrobial activity against both bacteria and fungi contamination. Furthermore, the concentration of a preservative in a formulation may determine if the antimicrobial activity is either cidal or inhibitory towards microorganisms. The minimum concentration for a preservative to prevent or inhibit the growth of a microorganism is known as the Minimum Inhibitory Concentration (MIC). The minimum preservative concentration for cidal activity against a microorganism is known as the Minimum Lethal Concentration (MLC). The MIC and MLC test data for a preservative is used to determine the spectrum of antimicrobial activity and use concentration in a product formulation. From this test data, it is important that the preservative in a formulation is at a sufficient concentration to have either cidal or inhibitory antimicrobial activity to prevent the growth or survivability of microorganisms in a product formulation.

Ingredients Affecting Preservatives

There are many raw ingredients that can affect the antimicrobial activity of preservatives. Besides a source of water, microorganisms need a source of microbial nutrients to proliferate in a formulation. With the presence of microbial nutrients, it could make a formulation more difficult to preserve because there is now an available source of nutrients for microbial proliferation and/or inactivate preservatives. There are also many raw ingredients that can give physical protection to microorganisms, chemically react, absorb or bind with a preservative to cause inactivation of the antimicrobial activity. Besides using preservatives to protect a formulation against microbial contamination, there are also raw ingredients that are able to enhance or increase the antimicrobial activity of a preservative system in a product formulation such as chelating agents, antioxidants, humectants, emollients, essential oils and fragrances.

Processing Conditions

The actual manufacturing conditions for a formulation need to be considered to have an adequately preserved product formulation. If the incorrect order of addition for a preservative is done during manufacturing, it may effect the partitioning of a preservative between the water and oil phases of an emulsion. The addition of emulsifying agents to a product suspension should always be performed first in order to coat the solid particles that would be able to absorb preservatives. Preservatives should always be added to a formulation at the appropriate pH to prevent decomposition or to have antimicrobial activity. The addition of a preservative to a batch at the incorrect temperature during processing may have an affect on the actual physical stability of a preservative in a formulation.

Packaging

The packaging in which a product formulation is going to be filled into is an important consideration when selecting a preservative system. There are many known examples in how the chemical composition of a product package will have an effect on the stability of the preservative system in a product formulation. The type of product package (e.g. tube, pump, aerosol can, jar) may pose an increase in the risk for the occurrence of microbial contamination to the product during consumer use by overwhelming the preservative system. A risk assessment needs to be performed to determine whether the configuration of the packaging can make an adequately preserved product formulation susceptible to microbial contamination during use by the consumer.

THE CHEMISTRY OF PRESERVATIVES

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A preservative is defined as a chemical that is added to a cosmetic to prevent the growth of or destroy microorganisms that may grow in the product. Various countries have different regulations of preservatives. Other speakers will cover this.

If you review the chemistry of the frequently used preservatives, you will find that they fall into these chemical classes: acids, aromatic alcohols, N-methylol containing substances; halogenated compounds, isothiazolinones, quaternia and 1,2 diols. Understanding the chemistry of these groups enables the formulator to choose the appropriate preservative with less guess-work and more science.

1. Acids

Organic acids have been used for preserving foods and cosmetics. These chemicals are active in the acid form but not the salt form:

$$\text{R-COOH} \rightleftarrows \text{R-COO}^- + \text{H}^+$$

As organic acids are weak acids, they exist in the acid or active form depending on the pH. The lower the pH the more activity.

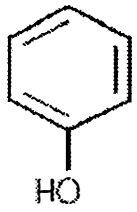
Typical acids and their dissociations are:

	pKa	% Active at each pH				
		3	4	5	6	7
Dehydroacetic	5.27	100	95	65	16	2
Benzoic	4.18	94	61	13	1.5	0
Sorbic	4.76	98	85	37	5.5	0
Salicyclic	2.97	48	9	1	0	0
Formic	3.75	85	36	5	1	0
Propionic	4.87	99	88	43	7	1

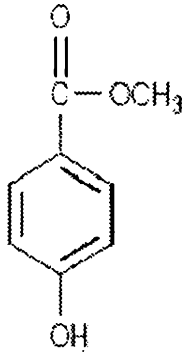
In general propionic, sorbic , salicylic and dehydroacetic acids are stronger against fungi, while formic and benzoic acids show better anti-bacteria action.

2. Aromatic alcohols

These represent the most important types of preservatives currently being used. All of these are substituted phenols. Phenol was once called Carbolic acid and found use as a hospital disinfectant. Trying to produce an odorless form of this, resulted in the discovery of parabens, our most frequently used preservatives. Like acids they are pH dependent:



Parabens are para hydroxybenzoic acid esters:



Parabens have limited solubility in water and this limits the quantity you can use.

Paraben	Water. 25°C	Water. 80°C	Propylene Glycol
Methylparaben	0.25	2.0	22
Ethylparaben	0.17	0.86	25
Propylparaben	0.05	0.30	26
Butylparaben	0.02	0.15	110
Benzylparaben	0.01	0.05	13

Parabens are strongest against Gram positive bacteria and fungi and are weakest against Gram negative bacteria. At a pH of 8 about half is dissociated into the inactive salt form.

Other phenolic preservatives include Phenoxyethanol and Benzyl Alcohol. These are much less active than parabens.

3. N-methylol containing substances

$$\text{N-CH}_2\text{-OH} \rightleftharpoons \text{N-H} + \text{HCHO}$$

This equilibrium between the secondary nitrogen and formaldehyde is dependent on the strength of this Nitrogen to Carbon bond. Many people refer to these as "formaldehyde" releasers.

The most frequently used members of this group are Imidazolidinyul Urea, Diazolidinyl Urea, DMDM Hydantoin, Sodium Hydroxymethylglycinate and Quaternium-15. They are all active against bacteria but are weak against fungi.

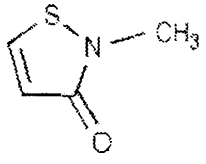
4. Halogenated compounds

The addition of a chlorine or iodine molecule to many compounds greatly increases their anti-fungal activity. Some of our most potent anti-fungal preservatives are of this group. They also typically have very poor water solubility and are difficult to incorporate.

In this class are Chloroxylenol, Chlorphenesin, Dichlorobenzyl Alcohol and Iodopropynyl Butylcarbamate.

5. Isothiazolinones

This class of compounds are some of the most potent preservatives and are frequently used at levels of less than 50 ppm.



There are three major isothiazolinones. Methylisothiazolinone, which was just approved in Japan and the EU; Benzylisothiazolinone which has not been approved ; and the mixture of Methylchloroisothiazolinone and methylisothiazolinone.

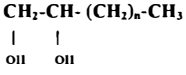
6. Quaternia

Quaternia compounds all have a positive charged nitrogen.

As a result these are pH dependent with the best activity at pH's above 7. They are weakest against Gram negative bacteria. They cannot be used with anionic systems. Popular preservatives in this class are: Benzalkonium Chloride, Benzethonium Chloride, Hexamidine Diisethionate, Polyamino Biguanide.

7. 1.2 Diols

There is growing interest in this class of preservative although none are registered as preservatives.



As the number of carbons increase, the solubility in water decreases and the anti-microbial activity increases. They are weakest against fungi. Most popular are the C-5.6 and 8. They are Pentylene Glycol, 1,2-Hexanediol and Caprylyl Glycol.

Conclusions

By looking at the structure of the preservative, a formulator can get some idea of its activity, its solubility and finally how it can be used to preserve formulations. When looking at preservative "cocktails", you should look to see if the components add to the overall activity or duplicate the activity of other parts.

REGULATORY UPDATE ON PRESERVATIVES - WORLDWIDE FOCUS

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Cosmetic chemists and their companies are constantly searching for a "worldwide product". It is an understandable goal that companies have a goal of having one version of a product worldwide. Whether or not this is possible, the first stumbling block for the formulator and microbiologist is the challenge of acceptable preservatives.

Companies are constantly in search of a "universal formula". Sometimes formulators will tell marketers that they have a "worldwide formula" for a product. When you hear this terminology, it is time for some additional research.

Each country in the world has its own regulatory system. Along with those systems come ingredient restrictions Preservatives are at the top of these lists.

Preservative lists fall into three categories:

1) Positive Lists
2) Negative lists
3) Restrictive Lists

Positive lists are those which communicate only which preservatives are acceptable. Negative lists are those which delineate preservatives that are not acceptable under any circumstances. Restrictive lists document which preservatives are acceptable, but only under certain circumstances. Often these are restrictions on percentage limits of use of the actual ingredient, or components of that ingredient, and types of use, such as leave-on products or rinse-off products

Preservatives are significant in that they have specific biological activity for their intended purpose. This functionality increases the scrutiny of the products, especially by governments and sometimes by consumer groups as well. These can often, rightly or wrongly, bear fruit in regulations that are designed to protect the health of consumers.

Many of the preservatives that are currently in use have been used for decades. With the advent of different scientific approaches, and history of use, some of these long-standing ingredients are under scrutiny.

The world is continuing to shrink. However, that does not mean that things are getting simpler. It only means that there is more information that is shared for the governments to analyze.

In the **U.S.** Regulatory landscape, FDA requires the producers of finished product to assure the products' safety for use by the consumers. This is true for Cosmetics and Over-the-Counter (OTC) Drugs.

In the **European Union**, there is a different approach. Acceptable preservatives are listed in a Positive list. This list is also a restrictive list, such that it defines certain use conditions for the ingredients. This can include specific products (for eye makeup only), product usage (not for use in products that are dispensed in aerosol form) and limits of usage in some or all products (e.g., 1% in rinse-off products, 0.5 % in leave-on products). Previously the E.U. has exercised the right to put preservatives on a "Provisionally allowed" list. There can also be a Negative list which provides for preservatives that are forbidden to be used in any type of Cosmetic product.

Of timely importance is the issue of safe use by the consumer by the use of date-identifying products. Most recently, the **European Union** enacted the Seventh Amendment to the Cosmetic Directive, which requires some kind of dating on almost all Cosmetic products. This new requirement, known as the **Period After Opening (PAO)** affects all

companies. Within those companies, those most directly affected are the Cosmetic Chemists and Microbiologists. The Period After Opening (strictly speaking, not an Expiration Date), is difficult to determine, yet this function of the Chemists and Microbiologists affects the entire company. With rapid product development timelines, this dilemma is intensified and is a challenge for all concerned. The parameters of determining the Period After Opening are many faceted. This requirement is completely new to our industry, and the implementation date was March 11, 2005.

Japan has its own set of regulations. They too, have a positive list of preservatives that can be used. If the preservative is not on the positive list, it may not be used to preserve Cosmetic products. Historically, Japan was one of the most difficult markets for preservative acceptance. The Ministry of Health and Welfare (now the Ministry of Health, Labor and Welfare), which is similar to our FDA, had a strict requirement for any ingredient to be approved before being used. For many years, Formaldehyde donor preservatives were not approved. Industry provided information citing the safety of these ingredients in other markets. The Ministry responded by noting their belief that the skin of the Japanese consumer was different and more sensitive than Caucasian skin. Therefore the safety of wide use in other markets was irrelevant for their safety concerns.

Some preservatives frequently used in the U.S. are unacceptable in other countries. They also sometimes have different limitations for use. Unfortunately, it is not until a new market is approached, that these differences come to light. Foresight and knowledge of the regulations can streamline the development process and can help eliminate additional formulation work.

When dealing with restricted amounts of ingredients, it is absolutely necessary to determine the presence and level of all preservatives in the finished product. Sometimes the presence of preservatives is overlooked, usually due to its presence in ancillary raw materials. Many companies have found that there are preservatives present in some raw materials, such as surfactants and liquid botanical extracts. These preservatives must be taken into account by virtue of their contribution to the finished product. If government authorities test the product off the shelf for preservative levels, they will be checking for all preservatives, regardless of the contributor.

Formulators are constantly faced with challenges choosing preservatives that are acceptable in International markets, not just in Japan, and in Europe, but in countries such as Australia, Korea and Taiwan. Becoming knowledgeable in the regulations affecting preservatives in various countries is imperative in designing formulas that will be acceptable in International markets. The definition of an International market must be carefully discussed. Often technical experts consider the U.S , Europe and Japan to be the only International markets. Other countries must also be considered when formulating.

Formulators and Microbiologists alike must now become experts in the field of Regulatory Affairs in order to stay competitive in our ever shrinking world.

HARMONIZATION OF MICROBIAL TESTING METHODS

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CTFA, through their microbiology committee, has been actively working with many groups to harmonize microbial quality and test methods. These include other organizations such as USP, COLIPA, JCIA and ISO TC-217 WG1. Recently harmonization on Microbial Limits Guidelines was agreed between the CTFA, COLIPA and JCIA.

Manufacturers of personal care products must assure that finished products are free from unacceptable levels and types of microorganisms. From the development of the product and optimizing the preservative system through raw materials testing, manufacturing, and product release testing, the microbiologist is involved. It has been generally accepted that the microbial limit test is a starting point of quality control for member companies; however they do not want to hamper the individualities of the cosmetic manufacturers by imposing numerical limits."

For the manufacture of personal care products that are also personal care products, the Japanese, European, and United States Pharmacopeias also supply tests. There has been an effort to standardize these methods, although the harmonized preservative effectiveness test differs in many details.

Trade organizations such as the Cosmetic, Toiletry & Fragrance Association (CTFA), the European Cosmetic, Toiletry and Perfumery Association (COLIPA), and the Japanese Cosmetic Ingredient Association (JCIA) work on behalf of member companies to standardize procedures, disseminate technical information on product use and safety, and interface with governmental/regulatory agencies to maximize compliance with the minimum amount of enforcement.

National and international standard setting organizations have also published preservative efficacy tests. The validity of the AOAC International method, based on the CTFA method, was the subject of a round-robin testing protocol. The ASTM method is under 5-year revalidation, and it is expected to be changed to bring it more into line with the CTFA method.

The International Standards Organization (ISO) has developed a number of microbiological methods that will soon be published as ISO international standards. Although the preservative efficacy test has been discussed, there is no formal movement to begin working on this new work area.

This presentation will review the existing methods for preservation efficacy, update the efforts at standardizing the CTFA/COLIPA/JCIA microbiological limit guidelines, and review the status of the ISO methods.

Microorganisms are opportunistic, and they may grow when nutrients (e.g., foods, drugs, cosmetics, raw materials, product residues, etc.), sufficient water, and environmental conditions are present. Products may provide sufficient nutrients for growth, and preservation is necessary for aqueous products in multiple-use containers. Microorganisms are ubiquitous and capable of adaptation and selection. No method can guarantee microbial control under all conditions.

The method for challenge testing involves the inoculation of the product sample with bacteria, yeasts and molds, some of which may either be pathogenic or cause spoilage, followed by testing the inoculated product for viability of the microorganisms at various periods as long as considered necessary. In some cases, these tests are supplemented by chemical tests to measure the amount of preservative in the product. These kinds of chemical tests are required for drug products in the US.

Test methods and guidelines, such as the CTFA *Guideline for Determination of Preservative Adequacy in Cosmetics*, provide guidance in many areas of PET testing. Some of the guidance includes:

- Personnel qualifications
- Importance of Manufacturing GMPs
- Factors to consider when designing preservative systems
- Importance of testing formulations during development
- Importance of using microbiologically acceptable raw materials
- Use of an unpreserved formula as a control to determine the need for preservation
- Stability testing of preservative systems
- Testing of product in final packages after storage

Similarly, the COLIPA *Microbiological Testing Guidelines on Microbial Quality Management* (MQM) note that the function of preservatives is consumer protection and prevention of spoilage during normal and reasonably foreseeable product use, that preservatives should not be used in lieu of good production hygiene, and that preservative selection should be chosen by microbiological challenge tests during product development. The Guideline states that packaging should be designed to restrict contamination and to avoid condensation of water from the product on the inner surface. Inactivation of preservative systems by the container and diffusion through it should be considered.

Unlike the CTFA Guidelines and the compendial methods, the Colipa *Guidelines* do not specify acceptance criteria. Thus, interpretation of adequacy of the preservative system is determined by review of the challenge test data, packaging, and intended consumer use.

The recommendations from each of the methods will be discussed, and compared, including the challenge organisms, media, differences, and acceptance criteria – both time of exposure and reductions observed – will be highlighted. The differences between "compendial" tests for drug products, standard test methods, and association guidelines will be reviewed. For example, the CTFA *Guideline* notes that the criteria are "minimal criteria". Manufacturers should understand that the preservative system of the formula, packaging, and consumer use must be evaluated in determining the preservative requirements of each product.

PRESERVATION - LEARNING FROM THE BASICS

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Preservative efficacy testing is performed on aqueous cosmetics and drugs to determine the minimum effective concentration of one or more preservatives required for adequate control of contamination. Products are satisfactorily preserved if they meet appropriate acceptance criteria and if they are packaged properly. This presentation discusses the advantages of the linear regression method, acceptance criteria, the preservative system concept, use of the principles of preservation to reduce the need for chemical preservatives, and the use of a miniaturized system for preservative efficacy testing without counting colonies.

Preservative Efficacy Test Methods

Several methods of preservative efficacy testing are used in different countries, including US Pharmacopoeia (USP), European Pharmacopoeia (EP), The European Cosmetic, Toiletry and Perfumery Association (COLIPA), Japan Cosmetic Ingredient Association (JCIA), and rapid procedures such as the Linear Regression Method.

Advantages of the Linear Regression Method

Several factors need to be considered when selecting the method of preservative efficacy testing used. These include reliability, time required for testing to be completed, labor and material costs, acceptance criteria, compliance with regulations or corporate guidelines, and environmental impacts of testing. The linear regression method offers several advantages, as follows:

Quantitative – The rates of microbial death are expressed numerically in D-values. The D-value is the time required for a 90% decrease (1-log reduction) in the population of test organisms. The rationale for use of D-values is that every organism has a characteristic rate of death when subjected to any lethal treatment.

Reliability – A method must be reliable or it is not worth using. The reliability of any test method depends on precision, sensitivity and accuracy. In microbiological testing, the precision generally depends on the skill and care used by the analyst. In the linear regression method, the correlation coefficient is used as an indicator of precision and is used to demonstrate the 'goodness of fit' of the data to the regression in this method. This allows a statistical control of each set of data. The linear regression method allows reliable determination of whether a product is satisfactorily preserved when appropriate recovery systems, culture conditions and acceptance criteria are used.

Acceptance Criteria – It s possible to set meaningful guidelines for accepting or rejecting a product based on the performance of its preservative system because the linear regression method provides a quantitative measure of the rate of die-off of test organisms. Selection of appropriate acceptance criteria is key to successful preservation of a product. Rigorous criteria generally are desirable because they minimize the likelihood of product contamination during manufacturing and during use by the consumer. They minimize the Phoenix Phenomenon (i.e., rebound of organisms after initial testing reveals no organisms present). A preservative system with D-values no greater than 30 hr (e.g., about a 6-log reduction in 7 days) for gram negative bacteria has a greater safety factor than one with D-values of 56 hr (e.g., 3-log reduction in 7 days). Target acceptance criteria for topical products are:

- D-value of ≤4 hr for pathogens/opportunistic pathogens
- D-value of ≤28 hr for non-pathogens, yeasts and molds
- Bacteriostatic or slowly bactericidal for *Bacillus* spp. spores.

Time Needed to Complete Testing – The linear regression method is rapid because it uses rigorous acceptance criteria. Testing for pathogens may be completed within three days; tests for nonpathogenic bacteria, yeasts and molds may be completed within two weeks.

No Rechallenge Testing – The rate of death (D-value) is independent of the initial concentration of test organisms present (up to the level at which the preservative system is overwhelmed). The same D-values were obtained on the first and 10th challenge of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in product samples. Elimination of rechallenge testing may reduce testing time by one week.

No Environmental Isolates – The use of environmental isolates of house organism is not necessary for routine testing. It is unlikely that anyone can find all of the environmental isolates that may occur when formulas are inadequately preserved, when compliance with GMPs is unsatisfactory, or when test methods are inadequate to control product release. If products meet appropriate acceptance criteria, they will kill house organisms grown on laboratory media.

Required D-value -- Three variables that determine whether a product will become contaminated are the preservative system of the formula, which may be expressed as the D-value, a packaging factor, and a consumer use/abuse factor. The required D-value (RDV) may be determined as follows:

$$RDV = \frac{\text{Maximum acceptable D-value for target organism}}{\text{(Packaging Factor) (Consumer Use/Abuse Factor)}}$$

Preservative System Concept

Preservative chemicals do not act independently in a formula because they are part of a "system". The preservative system includes preservative chemicals, the physicochemical composition of the product (pH, a_w, surfactants, chelating agents, fragrance chemicals, etc.), and protective packaging. Formula ingredients that have antimicrobial activity become "hurdles" for microorganisms.

Principles of Preservation

The principles of preservation are used throughout the food, drug and cosmetic industries. These principles are:

- Asepsis (keeping microorganisms out, microbial control of raw materials/processes, and protective packaging)
- Removal of microorganisms (e.g., washing, trimming, filtering to minimize microbial content)
- Retarding growth/killing of microorganisms using high/low temperatures, low and high pH, drying to achieve low water activity (a_w), removal of substrates and oxygen, use of preservatives or biocides, and irradiation or mechanical disruption.

The principles of preservation may be used to reduce and/or eliminate the need for addition of chemical preservatives in a formulation. Formulation components that contribute to the preservative system include compounds that raise/lower pH, compounds that lower a_w, lipids and esters, surfactants antioxidants, chelating agents, aroma chemicals, and botanicals. Self-preserving, or "preservative-free" products utilize the principles of preservation (including protective packaging) so that they are adequately preserved without use of chemical preservatives.

Summary

We will be able to build on the basics of preservation using the chemistry of preservatives, knowing the strategies of preservation, complying with regulatory requirements, and harmonizing test methods for global products. This information will enable us to select appropriate preservative efficacy test methods and acceptance criteria to ensure that our products are adequately preserved.

D&C BLACK #2

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Description:

Carbon Black is produced by a number of different processes, only 2 of which are relevant to the cosmetic industry, namely channel black and furnace black. Channel black is produced by the combustion of gas burned in iron channels, in which the pigment is deposited. Furnace black is produced by injecting oil into the flame zone of an enclosed reactor. It is used in cosmetics as a colorant.

Regulation History:

Prior to 1960, when the Color Additive Amendments were issued, carbon black of varying sources was used in foods, drugs and cosmetics. By the stipulation of the amendments, a color additive could only be permanently listed if its safety was proven under all conditions of use. However, it could be provisionally listed while testing was being conducted. All-gas channel black was selected to be provisionally listed while the industry gathered the required data, based on chemical and toxicological testing.

The major stumbling block to the approval of carbon black was the possibility that extractable polynuclear aromatic hydrocarbons (commonly referred to as PAH's or PNA's), particularly known carcinogens 3,4-benzpyrene and 1,2-benzantracine, might be present. Since no method for accurately determining the levels of these PNA's, at least not to the ppb level, existed at the time, the provisional listing was withdrawn. That was 29 years ago.

In 1982, the FDA "Constituents Policy" allowed that color additives containing trace amounts of carcinogens could be used, provided that the specific materials did not contribute and color function, and further provided that the color additive was shown to be non-carcinogenic by animal testing. In 1986, a petition was put forward by the CTFA, citing improved analytical methods, for the use of carbon black as a cosmetic color additive. Although various delays stalled approval, FDA finally approved the use of carbon black in 2004, although with certain provisions, specifically that the type of carbon black was limited to high purity furnace black, and that it should be subject to FDA certification to ensure compliance with the limits set forth in the final ruling. The approved name, reflecting the need for certification, was to be D&C Black #2.

D&C Black #2:

Carbon black, in order to be certified as D&C Black #2 for cosmetic use, is required to pass limit level tests for PAH's (benzpyrene and dibenzanthracene), as well as for the usual heavy metals. Surface area is also specified to correspond with a very small particle size, which would limit the potential for binding PAH's. The specifications are as seen in Table 1.

Specification		Limit
Surface Area (Nitrogen BET)		200 – 260 m^2/gm.
Weight Loss on Heating (950C for 7-min.)		2% max.
Ash		0.15% max.
Heavy Metals:	Lead	10-ppm max.
	Arsenic	3-ppm max.
	Mercury	1-ppm max.
Total Sulfur		0.65% max.
Total PAH's		500-ppb max.
Benzopyrene		5-ppb max.
Dibenzantracene		5-ppb max.
Total Color (as Carbon)		95% min.

Table 1. D&C Black #2 Specifications

Using D&C Black 2

The approved uses for carbon black for eyeliner, brush-on brow makeup, eye shadow, mascara, lipstick, blushers and rouge, makeup and foundation and nail enamel. Of course, due to the jet-black color of carbon black, the most popular uses will probably be in the area of mascaras and eyeliners, where the intensity of color obtainable will make it most desirable.

When carbon black was originally delisted, it was replaced by iron oxide black, which, by comparison, is less intense and less jet-black, often appearing gray or brown. Table 2 compares the properties of carbon black and black iron oxide:

Property	Carbon Black	Black Iron Oxide
Formula	C	Fe · FeO2
Particle Size	~ 20 – 30 nm	~ 300 nm
Particle Shape	Spherical	Cubic
Surface Area	200 – 260 m^2/gm	6.5 – 7.4 m^2/gm
Stability	Unaffected by heat, acid, alkali	Oxidizes at Temp 120C
Surface Chemistry	Hydrophobic	Somewhat hydrophilic
Oil Absorption	115-gm oil/100-gm pigment	32-gm oil/100-gm pigment
Water Absorption*	400-lbs/100-lbs pigment*	60-lbs/100-lbs pigment

* *The Chemistry and Manufacture of Cosmetics*, M deNavarre, ed, Wheaton, Ill: Allured publishing (1971). This value, from 30 years ago was specifically for carbon black, and may not apply in the case of D&C Black #2.

Table 2. Comparison of Carbon Black and Black Iron Oxide

The stability of carbon black is a great advantage over iron oxide. Often times, the heat generated by processing alone, especially in powder applications, using micronizers, would be enough to cause oxidation to the red form. Aside from the obvious aesthetic problem of shade, enough heat could be generated to cause cardboard drums, often used for pack-out, to smolder or burn. Due to its reactivity to heat, iron oxide black is classified as a hazardous material, and demands special shipping considerations. And, of course, the tinting strength of carbon black, at least 2 – 3 times that of typical black iron oxides is its biggest advantage.

Of course, there are disadvantages to the use of carbon black as well. Due to the fineness of particle size, it is very "fluffy" and can easily become suspended in the environment of the workplace if not handled with extreme caution. The high surface area makes it very adsorbent in most liquid applications, resulting in relatively high viscosity. In most carriers, it is difficult to obtain dispersions of more than 20 – 25%. Fortunately, the increased intensity allows for lower use levels, minimizing the viscosity increase experienced in applications like mascaras and eyeliners.

Conclusion

If interest in carbon black as a colorant can be maintained due to its advantages, it is possible that new wetting agents and/or surface treatments may, in time, be developed which reduce the difficulty of working with it. In any event, it is nice to see that the relisting of carbon black, even with the imposed restrictions, indicates that the delisting of a colorant may not mean that it is gone forever.

PERMANENT WAVING AND DEPILATION

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Introduction

Permanent waves and depilatories reply on the breaking of hair keratin disulfide bonds by mercaptans. Salts of thioglycolic acid (TGA) are most often used to achieve this purpose. From the standpoint of the chemistry involved the main differences between depilatories and perms are pH and counter ion and the fact that perms require reforming the S-S bond through an oxidative step(1). Perms using thioglycolate are typically formulated between pH 9 and 9.6. The so-called acid perms that utilize glycerol monothioglycolate (GMT) are typically adjusted to pH 6.8-7.0(2). The ammonium salt is most often used with TGA to provide swelling of the hair. Hydrogen peroxide is most commonly used for neutralization. Depilatories are typically formulated at pH 12-12.5 and utilize calcium or sodium salts or a mixture of the two(3).

Chemistry and Physics of Hair Reduction for waving and depilation

Wolfram and Underwood described the reactions between reducing agents and keratin disulfide bonds in detail(4). With TG the active species is the thiolate ion, RS-(1;4). With monothiol reducing agents the reaction proceeds in two steps converting two moles of the reducing agent to disulfide for each reduced S-S bond.

1.	Ker-S-S-Ker + R-S- ↔	Ker-S-S-R +	Ker-S-	
	Keratin	Mercaptan	Mixed disulfide	Reduced Keratin
2.	Ker-S-S-R +	R-S⁻ ↔	R-S-S-R +	Ker-S⁻
	Mixed disulfide	Mercaptan	disulfide	Reduced keratin

Reduction of the S-S bonds leads to structural changes that are keys to the action of perms and depilatories. Depilatory action is achieved when the hair is weakened to the point that it may be rubbed off of the skin. With perms the key is to mobilize the structure while the hair is under stress so that alterations in the polymeric structure can be locked in by the neutralizer treatment to produce permanent set. This is achieved by breaking a fraction of the disulfide bonds and assisted by the presence of free SH groups in the hair which can participate in the sulfide-disulfide interchange reaction(1;5).

It can be argued that the key bonds that must be broken for either depilation or permanent waving are those that support tensile stress when the hair is under load(6-8). This makes chemical stress relaxation methods very useful for studying reduction kinetics related to either process(6;8-12). To use these methods a hair is placed under tension in buffer and stress relaxed until it supports a constant level of force. The buffer is then switched for the reducing agent and the decay of the force with time is followed. This method, also called Single Fiber Tensile Kinetics (SFTK) has been used to study the effects of pH, temperature and hair type on the reaction kinetics(6) and to study the effect of reducing agent structure. (6;13) on reduction rates. I have postulated that the reaction kinetics follow at least two different mechanisms depending on pH and reducing agent used(7;11). For example with TGA reaction kinetics apparently follow a pseudo first order mechanism at low pH while at higher pH reaction proceeds by a sharp front or "moving boundary".

The SFTK method has been applied to the study of depilatories as well as perms. For depilatories the time required for the tensile force to decay to 5% of its original value was found to be a convenient indicator of depilatory activity.(11) that correlated well with depilation times measured *in vivo*. Effects of pH, counter ion and pretreatment were quantified. Beidman(14) used a thermomechanical analyzer to determine the time required for the stretching of a hair bundle in depilatory solution to begin and also reported good correlation with in vivo depilatory efficacy.

We have also compared SFTK data to data obtained using amino acid analysis for TGA and cysteamine(9) and concluded that while SFTK kinetics appear somewhat faster than kinetics determined by amino acid analysis the rank order of the reaction is consistent between the two methods.

Wortmann and Souren(12) used an innovative combination of chemical stress relaxation and intermittent strain pulses to try to correlate changes in tensile properties with the level of permanent set developed. The reaction was followed during both the reduction and oxidation steps. While the relaxed tensile force will not increase during oxidation the increase in modulus measured from the small strain pulses will be dependant on the number of S-S bonds reformed. The set recovery of hair subjected to the same treatment protocol was measured by treating the hair on a small cylinder of known diameter D_c, then measuring the diameter, D to which the hair loop opened in water. They reported that recovery, R, the opposite of set, is directly proportion to the ratio of the fraction tensile strength lost during the reduction step to the fraction of tensile strength regained during the oxidation step.

The efficacy of perms may also be evaluated using the pegboard (15) or the test tube curl method(15-17). While direct kinetic data can't be obtained from these methods they are very useful for laboratory evaluation of permanent waves.

Unfortunately the waving process does do some damage to hair. A small but significant reduction in tensile strength occurs(2;18;19). Recent results by Nishikawa et al indicate that the content of α-helical protein is reduced from 23.7% to 20.4% and that the resultant conformational change is to random coil.

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A Novel Phosphate Ester for Hair-Coloring Enhancement

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Introduction

Bleaching and coloring of hair has become increasingly popular over the past years. Color is produced inside the hair fiber by hydrogen peroxide–induced oxidation and coupling reactions of aromatic amines and phenols. Since hydrogen peroxide is used, it will concurrently bleach the hair's melanin, and shades lighter than the natural hair color can be obtained. Oxidative dyeing is usually carried out under alkaline conditions preferably at pH 9 to 10. At the time of dyeing, the alkaline solution containing the dye components is mixed with the peroxide developer, which is stabilized for storage at a pH of 3 to 5. The resulting mixture is then applied to hair for 20 to 40 minutes before being rinsed off with water.

Surfactants play important roles in the efficacy of hair color products:

- Emulsification – to form stable and fine O/W emulsions for better solubility and distribution of dye intermediates and coupling agents
- Wetting – to accelerate the diffusion of dye intermediates and coupling agents into hair cortex
- Cleaning and Spreading – to ensure uniform dye coverage on the air surface and help removing dye residue on the hair surface, which contributes to a poor feel, dull appearance, and difficult combing.

There have been a lot of studies on the factors that affect the bleaching and coloring process. These factors include dye solubility and distribution, coupling rates between dye and coupling agent, diffusion rates of dye intermediates and coupling agents into the hair cortex, hair swelling rate, effect of different alkali on dyeing process, and the efficiency of dye use. Phosphate ester compounds have long been used in hair and skin care formulations including hair relaxers, hair perms, sunscreens, and color bases as excellent emulsifiers and viscosity thickening agents. In this article we report our recent findings on how phosphate esters affect oxidative dye coloring performance. An excellent Hair Color Enhancer (HCE) - Oleth-5 Phosphate and Dioleyl Phosphate has been found.

Experimental

Materials

1. Hair Dye bases were obtained from the Applications Lab at Innovation Center of Croda Inc
   - Color Base A – A regular oxidative auburn color base without phosphates
   - Color Base B – A modified Color Base A with addition of 4% HCE
2. Hydrogen Peroxide Hair Color Developer was prepared by Application Lab of Croda Inc
3. Bleached Hair was purchased from International Hair Importers and Products, Inc, New York

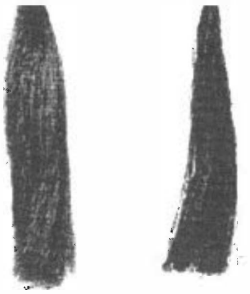
Measurements

1. Hair color indexes (L, a*, b*) were determined using a LabScan XE Spectrocolorimeter equipped with a special sample holder for hair tress.
2. Dynamic advancing contact angle at the interface of hair fiber/deionized water was determined using a Cahn DCA-315 Dynamic Contact Angle Analyzer at constant temperature of 25°C.
3. Emulsion structure was inspected under a Nikon Optiphot –Pol Microscope, and the image was saved and analyzed by image analysis software.
4. Viscosity profile of emulsion samples was measured using a Brookfield DV-III Rheometer at constant temperature of 25°C

Results and Discussion

1. Change in Hair Color with Time

The photos of dyed hair samples are shown in Figure 1. The determined changes in hair red color index, a*, of bleached hair before and after dyeing with the two different dye bases are presented in Figure 2. It can be easily seen by visual inspection that the hair dyed with the color base containing HCE showed richer red color than the hair dyed with a regular base. The determined changes in the red color index of Δa^*, in yellowing index of Δb^*, and in total color difference of ΔE of these hair tresses clearly indicated that the development rate of red color on the hair dyed with the color base containing additional HCE was faster than that on the hair dyed with the base without HCE. This experimental result indicates that addition of HCE into regular oxidative auburn color base not only produced richer final shade, but also accelerated the whole coloring process and demonstrated faster coloring rate compared to the hair dyed with the corresponding regular dye without HCE.



Dye base Dye base with HCE

Figure 1 Photo of dyed hair tresses (20')

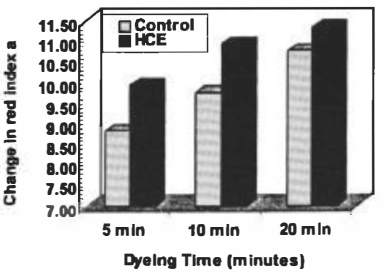
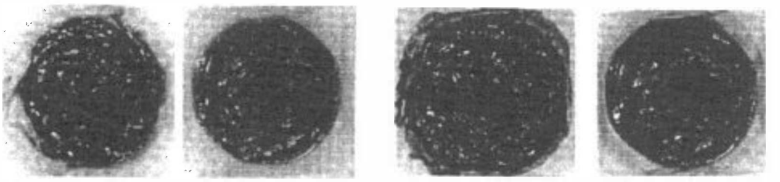


Figure 2 Change in red index of dyed hair

2. Change in The Color of Coloring Mixtures

In order to understand the mechanism of enhancement in coloring performance by addition of HCE in the color base, the color development process of mixtures of color base and the developer at different mixing time was investigated. It is obseryed that the initial color development rate in the mixture of the color base containing HCE was slower than that of the mixture without HCE. Typical photos at 2 and 10 minutes of mixing time are presented in Figure 3.



Control Base (2') HCE Base (2') Control Base (10') HCE Base (10')

Figure 3 Change in color of mixtures of color bases and developer at different mixing time

It is clear that the color development in the mixture containing HCE appeared lighter in the emulsion compared to the emulsion without HCE. This could mean that the dye intermediates and coupling agents were protected in the emulsion at the beginning, which would allow the individual components to remain separated until diffuse into the hair. Therefore, the hair dyed with the hair color containing HCE could allow more of the dye intermediate and coupling agents to diffuse into the hair cortex, react inside the hair, thus locking in more color rather than laying on top of the hair. As a result, this may explain why the hair dyed with the hair color containing HCE showed a much better color uptake, fast coloring rate, and final richer color inside the hair cortex.

3. Change in Emulsion Structure of Coloring Mixtures

It is observed that the color mixture (emulsion) with HCE showed more uniformly dispersed oil droplets, smaller average droplet size, and faster oil droplet-breaking rate compared to the regular color mixture without HCE. Since most of dye intermediates and coupling agent are oil-soluble and prefer to stay in the oil phase, smaller number of oil droplets, more dye intermediates and couplers are distributed outside oil droplets and less chance to interact each other to form complexes outside the hair after oxidation. This means that when the color mixture (emulsion) was applied on hair, dye intermediates and coupling agent in the HCE color mixture had more and better chance to contact with and diffuse into hair, and therefore to enhance the coloring performance.

4. Change in pH and Viscosity of Coloring Mixtures

Experimental results also indicated that the color mixture (emulsion) with HCE showed slower increases in pH value and viscosity in the initial stage of mixing (less 10') compared to the regular color mixture without HCE. These two factors are also favourable in the diffusion of un-reacted dye intermediates and coupling agents into the hair cortex.

In Vitro Dermal Absorption and Metabolism of D&C Red No. 17

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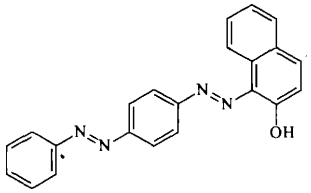
Introduction

D&C Red No. 17 is approved for use in externally applied drug and cosmetic applications, in amounts consistent with good manufacturing practice. Possible cosmetic uses of D&C Red No. 17 include skin and hair care preparations and suntan products (1). Concerns about the safety of this color additive (1-[4-phenylazophenylazo]-2-naphthol (PAN) is the primary color constituent) have been raised due to potential metabolic cleavage of PAN to yield 4-aminoazobenzene. It was therefore of interest to examine the skin penetration of PAN and to determine if this compound is metabolized in viable skin.

Figure 1: Structure of PAN, the primary color constituent of D&C Red No. 17



Methodology

In vitro skin absorption studies were conducted in flow-through diffusion cells as previous described (2). Studies were conducted with either freshly obtained viable pig skin or human cadaver skin. The skin was dosed with a commercially available sunscreen product that contained D&C Red No. 17 (15 µg/ml) that was spiked with a tracer amount of ^{14}C-PAN.

PAN metabolism in viable porcine skin was investigated using HPLC methods. Samples of homogenized viable porcine skin were extracted with ethyl acetate (1:2 vol:vol), which was separated from the aqueous layer and concentrated under a N_2 stream.

Results

Only a small amount of ^{14}C-PAN was absorbed through human and porcine skin in 24 h (Table 1). Only 0.05% of the applied dose was found in the receptor fluid with human skin. The time course of absorption shows similar absorption between human and pig skin with a significant increase in pig skin absorption in the 24 h sample. Most of the PAN penetrating into the skin remained in the skin. Total penetration was similar in human (10.5 %) and porcine (13.2 %) skin.

Table 1: PAN penetration in human and porcine skin after 24 hours (% total applied dose)

	Human	Porcine
Receptor fluid	0.05±0.01	0.53±0.15
Skin content	10.5±1.7	12.6±1.2
Total applied dose penetrated	10.5±1.7	13.2±2.1
Wash	91.8±11.7	91.6±4.7
Recovery	102.4±11.1	104.2±4.9
Values are mean ± SEM for human (n=7) and porcine (n=16) skin		

Because of the large amount of PAN found in the skin at 24 h, extended studies were conducted for 72 h (with a skin wash at 24 h) to determine if additional PAN would be absorbed (Table 2). Receptor fluid levels increased slightly with human skin and were now similar to pig skin values. Porcine skin levels of PAN were unchanged when 24 and 72 h values were compared. Human skin levels also did not decrease during the extended study. It appears that the levels of PAN penetrating into the skin are unable to readily diffuse out of the skin into the receptor fluid even in the extended absorption study.

Table 2: PAN penetration in human and porcine skin after 72 hours (% total applied dose)

	Human	Porcine
Receptor fluid	0.30±0.04	0.45±0.15
Skin content	17.1±4.4	12.2±2.2
Total applied dose penetrated	17.4±4.4	12.7±2.2
Wash	91.5±6.7	96.9±7.8
Recovery	108.9±7.6	109.5±9.3
Values are mean ± SEM for human (n=8) and porcine (n=4) skin		

Metabolism of PAN could only be examined in extracts from pig skin samples since the levels of radioactivity that were absorbed into the receptor fluid were extremely small. In spite of the fact that large amounts of radioactivity were present in the skin at 24 h, no metabolites of PAN were detected by HPLC analysis. Metabolism of PAN to 4-aminoazobenzene in skin would require the cleavage of an azo bond. We have previously shown that several other azo colors were significantly metabolized with azo bond cleavage in mouse, hairless guinea pig and human skin (3). However, these colors had better water solubility and were more readily absorbed than PAN. Human skin samples in the current study were not analyzed for metabolism since they were from non-viable cadaver skin.

Conclusion

The skin absorption of ^{14}C-PAN into the receptor fluid beneath the skin was significantly less than 1% with either human or pig skin in both the 24 and 72 h studies. Substantial amounts of ^{14}C-PAN (10-17 %) penetrated into human and pig skin in 24 hr. Pig receptor fluid levels of PAN did not increase in the extended absorption studies suggesting that material in the skin may not be available for systemic absorption. Human receptor fluid levels of PAN did increase significantly at 72 h but the 72 h values were still low and similar to those with pig skin. No metabolism of PAN to 4-aminoazobenzene was observed in viable pig skin.

References

1. Gottschalck, T. E. and McEwen, G. N., *International Cosmetic Ingredient Dictionary and Handbook, 9th Ed.*, The Cosmetic Toiletry and Fragrance Association, Washington DC, *2004*.

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