In vitro/in vivo and analytical evaluation of sunless tanning formulations containing different rheology modifiers

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Synopsis

In vitro data suggest that different *in vivo* performances are expected for two dihydroxyacetone (DHA)containing formulations with similar concentrations of DHA and excipients but different commercially available rheology modifiers: one with a cationic polymer-based rheology modifier (blend) [dimethylacrylamide/ethyltrimonium chloride methacrylate copolymer (and) propylene glycol dicaprylate/dicaprate (and) PPG-1 trideceth-6 (and) C10-11 isoparaffin]; and the other with a polyacrylamide-based rheology modifier (blend) [polyacrylamide (and) C13-14 isoparaffin (and) laureth-7]. Both rheology modifiers (blends) contained comparable levels of polymers and were used at 3% w/w (as supplied). Differences in color development were illustrated *in vitro* with respect to the yellow/red and lightness/chroma parameters, which were confirmed in the followup *in vivo* studies. The test article with the cationic polymer-based rheology modifier produced a more natural sunless tan, comparable to a desirable sun-induced tan, for all panelists, one that was more uniform and lasted longer compared with the sunless tan generated by the test article with the polyacrylamidebased rheology modifier. A method for HPLC analysis of DHA in sunless tanning formulations was established and utilized to confirm concentrations of DHA in test articles.

INTRODUCTION

A tanned appearance is considered a symbol of a healthy and active life. A sunless tan is generated by the Maillard reaction of DHA and/or erythrulose with the amino acid groups in peptides and proteins in the stratum corneum. DHA is a simple three-carbon *keto*-sugar obtained by fermentation of glycerin. The Maillard reaction, first described in 1912 by Louis-Camille Maillard, occurs between sugars and amino acids, peptides and proteins, and produces dark pigments called melanoidins. Sunless tanning products contain DHA in concentrations ranging from about 1.25% to 15%. Most drugstore products range from 3% to 5%, with professional products ranging from 5% to 15%, corresponding to product coloration levels from light to dark. The sunless tan usually takes two to four hours to appear on the skin surface, and continues to darken for 24 to 72 hours, depending on formulation type. DHA does not damage the skin, and is considered a safe skin-coloring agent. DHA-based sunless tanning has been recommended as a safer alternative to sun exposure by the Skin Cancer Foundation, the American Academy of

Dermatology Association, the Canadian Dermatology Association, and the American Medical Association. Mintel says in its recent report that users of sunless tanning products in the U.S. are more receptive to new products compared with users of more mature personal-care categories and that a new entrant that produces significantly better tanning results could make a significant dent in the position of leading brands. Increasing awareness of the health risks associated with sun exposure motivated 39% of those surveyed to try sunless tanners. Among those consumers who have stopped using sunless tanners, 42% gave the reason that the products are too hard to apply, while 33% cited the products' "artificial" appearance (1). Different skin types may react differently with DHA due to the individual amino acid content, moisture level, skin tone, pH, and thickness. The result could be an uneven tan, one that is too dark or too light, or an orange color. It is known that various chemicals can modify or enhance the tanning reaction obtained with DHA on skin. Examples of such ingredients include amino acids (2), amino-substituted silicone compounds (3), polyacrylamide (4), amphoglycinate (amphoacetate) derivatives (5), thickeners, humectants, UV filters, vitamins, and emollients (6), and strong antioxidants (7). Certain thickeners, such as carbomer-type polyacrylates, when combined with DHA produced malodor and/or browning of the composition (4). However, the information regarding the impact of rheology modifiers on the development of sunless tan in vitro/in vivo and the analytical methods for DHA analysis in finished goods formulations is limited.

Our objectives in this study were:

- To evaluate and compare the ability of two DHA-containing sunless tanning formulations with similar excipients but different rheology modifiers (blends), one with a cationic polymer-based rheology modifier and the other with a polyacrylamide-based rheology modifier, to influence the sunless color development *in vitro* and *in vivo*
- To establish an analytical method to determine DHA concentration in sunless tanning formulations.

MATERIALS AND METHODS

TEST ARTICLES

Rheology modifiers were incorporated at 3% w/w levels (as supplied) in test formulations X and K, containing similar concentrations of DHA and the excipients:

- Test article X with cationic polymer-based rheology modifier [INCI: dimethylacrylamide/ethyltrimonium chloride methacrylate copolymer (and) propylene glycol dicaprylate dicaprate (and) PPG-1 trideceth-6 (and) C10-11 isoparaffin], recently introduced to the market by Ciba Corporation (part of BASF Group).
- Test article K with polyacrylamide-based rheology modifier [INCI: polyacrylamide (and) C13-14 isoparaffin (and) laureth-7], from Seppic.

The concentrations of active polymers are comparable in both commercial rheology modifiers (blends). The polyacrylamide-based rheology modifier utilized in test article K was selected for this comparative evaluation because it was successfully used in sunless tanners demonstrating good efficacy (4). Formulations of the test articles are presented in Table I.

SUNLESS TANNING FORMULATIONS

IN VITRO/IN VIVO EFFICACY EVALUATIONS—GENERAL APPROACH

An *in vitro* efficacy testing methodology for evaluation of sunless tanners described by Jermann *et al.* (6) with our modifications (7) was utilized; pre-hydrated Vitro Skin[®] (N-19) (8) was used as a substrate. This *in vitro* methodology is a reliable tool to predict the efficacy and differences of self-tanning formulation performance on human skin (5–7). For the analysis of skin color *in vivo* after the application of test articles, the "natural universe of suntan" and "natural universe of suntan tonality" realms were from Muizzuddin *et al.* (9), describing "…a cluster plane encompassing the distribution of normal skin tanning color representing the 'natural universe' of skin tanning or a response region within which natural skin tan color was observed."

PROCEDURE-IN VITRO

The substrate was pre-cut into 4-cm by 4-cm pieces and pre-hydrated according to IMS-USA protocol (8). The application dose was 2 mg/cm^2 ; temperature: $76^\circ-78^\circ\text{F}$. Each piece of substrate was prepared for the experiment by uniformly applying 0.032 grams (2 mg/cm²) of test article on the surface with a pre-saturated finger cot. The substrate was then placed in a slide frame and put in the color development chamber. Three slides for each test article were used. The test articles were coded and their compositions were revealed after the *in vitro* study was completed. The colors of the samples were evaluated every 24 hours (three measurements per slide) for four days with a ColorTec-PSMTM Colorimeter: Observer 10°; primary illuminant D65; CIE (1976) L*a*b* color space with tri-stimulus color values: L* (lightness), a* (red-green axis), and b* (yellow-blue axis). The difference in C* (chroma/saturation) is calculated according to the following equation:

$$dC^* = \sqrt{(da^{*2} + db^{*2})}$$

Table I
Description of Test Formulations: X(with cationic polymer-based rheology modifier) and K (with
polyacrylamide-based rheology modifier)

	%	w/w
Ingredient: INCI name	X	К
Water	89.65	89.65
Pentylene glycol	4.00	4.00
Dihydroxyacetone	3.00	3.00
Phenoxyethanol (and) methylparaben (and) ethylparaben (and) butylparaben (and) propylparaben (and) isobutylparaben	0.35	0.35
Dimethylacrylamide/ethyltrimonium chloride methacrylate copolymer (and) propylene glycol dicaprylate/dicaprate (and) PPG-1 trideceth-6 (and) C10-11 isoparaffin	3.00	_
Polyacrylamide (and) C13-14 isoparaffin (and) laureth-7		3.00
Sodium hydroxide, 10% aqueous solution pH	q.s. 3.90	q.s. 3.76

The total difference between two colors in CIE space is described as ΔE^* ab and provides an integrated measurement of both chroma and lightness/darkness changes:

$$dE * ab = \sqrt{[(dL^{*2}) + (da^{*2}) + (db^{*2})]}$$

PROCEDURE-IN VIVO

In vivo studies were conducted on five panelists using the volar aspects of their forearms as application sites. Application areas were 50 cm^2 . Before application of the test formulations, all panelists washed their forearms with Dove bar soap, rinsed with water, and patted their forearms dry with paper towels. A period of 15 minutes was allowed before conducting the initial measurement of the skin color on marked application areas (five measurements per area) using the ColorTec-PSM Colorimeter. The test articles were coded to hide their identity from those used in the *in vitro* studies, and their compositions were disclosed after the *in vivo* study was completed. Each test article was applied once on each panelist. The application dose was 2 mg/cm², with an exact application amount of 0.1 g per application area. Left and right forearms were alternated to randomize the application sites for the test articles. The products were applied using finger cots pre-saturated with test article. Color measurements were taken at 24, 48, and 120 hours after a single application. Between measurements panelists were asked to follow their regular hygiene routine, taking showers, washing hands, etc.

MATERIALS AND EQUIPMENT USED IN THE IN VITRO/IN VIVO STUDIES

- Vitro-Skin[®] (N-19) (Lot# 8302), foam block and glassless slide mounts from IMS, Inc.
- Powder-free class 100 finger cots (Lot# FOY8) from Fisher Scientific
- ColorTec-PSM Colorimeter from ColorTec.

HPLC METHOD

An analytical method for the determination of the concentrations of DHA in sunless tanning formulations was developed based on the method described by Biondi *et al.*, HPLC analysis of a pentafluorobenzyloxime derivative (10) with our modifications.

Sample preparation for HPLC. The samples were prepared for analytical testing by combining 0.25 grams of each sample with 5 ml of sodium chloride (NaCl)-saturated aqueous solution, followed by vortex mixing for one minute. Then 5 ml of water was added to each sample and stirred for six hours. After that 25 μ l of the sample and 50 μ l of derivatizing agent O-(2, 3, 4, 5, 6-pentafluorobenzyl) hydroxylamine hydrochloride 98% (Aldrich, 194484) were pipetted into a 10-ml volumetric flask and diluted using 50:50 acetonitrile and water.

The standard used was DHA, cosmetic grade, from Napp Technologies LLC (Lot LL07-2046). The preparation consisted in dissolving 0.0330 grams of the reagent in 10 ml of

citrate buffer. The citrate buffer was prepared by adding about 0.9 grams of the citric acid to 100 ml of water, titrated to pH 4.0 using a 0.1 N aqueous solution of sodium hydroxide (NaOH).

Standard preparation for known in-house formulations. A base was provided and used to spike with a known amount of standard, used for the standard curve. The base was treated like a sample that was stirred and agitated with NaCl-saturated aqueous solution and water for six hours and then spiked with a known concentration of the standard. A standard linear curve was prepared as follows: 0.0895 grams of the standard was weighed in a 50-ml volumetric flask, 10 ml of the saturated solution of NaCl was added, and the remaining volume was filled with water. Amounts of 2 μ l, 5 μ l, 10 μ l, 15 μ l, and 20 μ l were pipetted into 20-ml scintillation vials. Fifty microliters of derivatizing reagent was added. Then 10 ml of an acetonitrile:water (50:50) solution was added to the scintillation vial. The derivatization reaction was completed after five minutes at room temperature.

HPLC parameters. An Agilent 1100 series HPLC with a quaternary pump and UV/Vis DAD detector with a detection wavelength at 262 nm was used. The column utilized was a Discovery 5- μ m C18 column, 25 cm × 4 mm, from Supelco (Cat.# 504971-40), with an injection volume of 35 μ l. The flow rate was set a 1 ml/min at 45°C. The run time was 25 min. An acetonitrile: water (50:50) solution was the mobile phase (isocratic). The retention time for the DHA-derivatized complex was 5.3 min.

ANALYTICAL RESULTS AND DISCUSSION

This HPLC method works well with the known sample matrix base. Our modifications of the method of Biondi *et al.* (10) included the following changes: a matrix base (placebo) and samples were pre-mixed with an NaCl saturated solution and water, and stirred for six hours. The base matrix was spiked with a known amount of standard (DHA) and used for quantitation. It is advantageous to prepare the standards in a known matrix since it eliminates the possibilities of interference from that matrix. This method also gives reliable data for similar types of commercial formulations. A liquid–liquid extraction by dichloromethane of the commercial samples, for which the base matrix is not available, can be effective for free DHA extraction and the removal of base ingredients (10). The column temperature for the analyses was increased to 45°C for an improved peak shape of the DHA component. The HPLC chromatograms are presented in Figure 1, the results of DHA analysis in test articles are depicted in Table II, and a correlation between the DHA added to the base matrix and the DHA found is shown in Table III.

IN VITRO EXPERIMENTAL DATA AND DISCUSSION

In vitro experimental data are presented in Figures 2-4 below. As shown in Figure 2, both test articles showed similar trends in total color (ΔE^*ab) changes vs time. Figure 3 shows the differences in the yellow (Δb^*) and the red (Δa^*) values after 72 hours of test article application. According to Jermann *et al.* (6), this particular time interval provides good correlation between relative tanning responses *in vitro/in vivo*. Noticeable variations in color depending on the test article were observed. Yellow/red balances varied, depending on the test article: X generated more red/same yellow color compared to K. As illustrated in Figure 4, ΔL^* values after 72 hours were darker with the same ΔC^* values X vs K. *In*



Figure 1. HPLC chromatograms.

Table II Analytical Results

Test article	DHA (%)	
X K	3.25 3.18	

 Table III

 HPLC Method Validation: DHA Recovery

Sample	DHA added	DHA found	% RSD	% Recovery
A	0.000	0.000		
В	1.000	1.049	3.376	104.892
С	1.997	2.093	3.307	104.788
D	2.997	2.930	1.597	97.767
Е	3.994	4.120	2.189	103.144
F	4.993	5.096	1.443	102.062

vitro data suggested that different *in vivo* performances are expected for X and K, especially with respect to the yellow/red and lightness/chroma parameters of the skin color.

IN VIVO EXPERIMENTAL DATA AND DISCUSSION

The *in vivo* part of this study was conducted on five panelists and focused on the application, results, and measurements related to test articles X and K. Four panelists were categorized based on their ITA° values (Table IV), according to Chardon *et al.* (11), as very





Figure 2. Impact of the test articles on ΔE^*ab *in vitro* vs time.



Figure 3. Impact of the test articles on the color response in vitro at 72 hours.

light to light and one panelist was categorized as intermediate. *In vivo* experimental data are presented in Figures 5–7.

Panelists noticed a slightly less intense color development after application of X within the first 24 hours, but the color of the tan was more even throughout the application area compared to K. These visual observations were confirmed instrumentally (Figure 5).

The average standard deviation of ΔE^*ab for all panelists at 24 hours was 4.2 times higher for K than for X. At 48 hours the average of ΔE^*ab was now slightly higher for X than K, and it was still higher after 120 hours. Apparently the fading of the sunless tan occurred at a slower rate with X than with K. Color associated with X was still visible with the "naked eye" after 120 hours for four panelists and for one panelist with K. This

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Figure 4. Changes in ΔC^* and ΔL^* values *in vitro* at 72 hours. ΔL^* values indicate the "darkening" trend.

Table IVITA° Values of Panelists					
	ITA° values*				
Panelist	Left forearm	Right forearm	Skin color categories		
1	52.86	51.71	Light (>41 to 55)		
2	52.48	55.74	Light (>41 to 55) to very light (>55)		
3	50.47	55.12	Light (>41 to 55) to very light (>55)		
4	53.01	51.75	Light (>41 to 55)		
5	39.78	40.55	Intermediate (>28 to 41)		

TA = [Arc tangent ((L*-50)/b*)] 180/3.1416.

indicates potentially better longevity of the tan generated by X vs K. *In vivo* data were also plotted in the respective figures below. Figure 6 illustrates "the natural universe of suntan tonality" (9) via the balance between changes in the yellow and red components of the natural suntan. The natural universe of suntan tonality determines how "natural" the tonality of a sunless tan appears relative to a truly natural, sun-generated tan. All measurements associated with X were within the "natural universe of suntan tonality" range, providing that the sunless tan generated by X is comparable to a natural sun-induced tan for all panelists (Figure 6).

Figure 7 shows the balance between changes in Chroma, ΔC^* , and in reflectance, ΔL^* , induced by the test articles in the realm of the "natural universe of suntan" (9) for sun-induced tan. Overall, X produced a sunless tan color consistent with the "natural universe of suntan tonality" range, except for one panelist, whose color was outside the range, but the rest of the panelists had close color responses and were well within the range (Figure 7).

Application of K resulted in three panelists being outside the "natural universe of suntan tonality" range (Figure 6). Two of the panelists developed a color that was more yellow than



 ΔE^*ab Color Development, Average ΔE^*ab over time

Figure 5. Impact of the test articles on ΔE^*ab values *in vivo* vs time.



Figure 6. Impact on color response in vivo at 24 hours.

natural suntan, while another panelist seemed to have less red than natural suntan. The tonality of the sunless tan generated by K was less "natural" since three out of five panelists were outside the "natural universe of suntan tonality" range. K also produced the following results based on ΔC^* and ΔL^* measurements: one of the five panelists was outside the "natural universe of suntan tonality" range and another panelist had a less developed tan that was on the borderline of the range (Figure 7).



Figure 7. ΔC^* and ΔL^* values *in vivo* at 24 hours after application.

CONCLUSIONS

In vitro data suggest that different in vivo performances are expected for two dihydroxyacetone (DHA)-containing formulations with similar concentrations of DHA and excipients but different commercially available rheology modifiers: one with a cationic polymer-based rheology modifier (blend) [dimethylacrylamide/ethyltrimonium chloride methacrylate copolymer (and) propylene glycol dicaprylate/dicaprate (and) PPG-1 trideceth-6 (and) C10-11 isoparaffin]; and the other with a polyacrylamide-based rheology modifier (blend) [polyacrylamide (and) C13-14 isoparaffin (and) laureth-7]. These rheology modifiers were used at 3% w/w (as supplied) and contained comparable concentrations of active polymers. In the followup in vivo studies, the test article with the cationic polymer-based rheology modifier produced a more natural sunless tan, comparable to a desirable sun-induced tan, for all panelists; the sunless tan was also more uniform and lasted longer compared with the sunless tan generated by the test article with the polyacrylamide-based rheology modifier. Panelists noticed a slightly less intense color development after application of X within the first 24 hours, but the color of the tan was more even compared to that from K. These visual observations were confirmed instrumentally: the average standard deviation of ΔE^*ab for the panelists after 24 hours was 4.2 times higher for K than that for X. At 48 hours the ΔE^*ab vs time trend was reversed, with slightly higher values for X than K. Apparently the fading of the sunless tan occurred at a slower rate with X than with K. Color associated with X was still visible with the "naked eye" after 120 hours for four panelists and for only one panelist with K. This indicates potentially better longevity of the tan generated by X compared to K, which may be attributed to the presence of the cationic polymer-based rheology modifier (blend) in X. Overall, X produced a sunless tan color more consistent with the "natural universe of suntan tonality" range, except for one panelist whose color was outside the range, but four other panelists had similar color responses and were well within the range.

As for the "natural universe of suntan tonality," measurements associated with X were within this realm, indicating that the sunless tan generated by X is comparable to a desirable natural sun-induced tan for all panelists. Application of K resulted in three panelists being outside the "natural universe of suntan tonality" range. Two of the panelists developed a color that was more yellow than natural suntan, while another panelist seemed to have less red than natural suntan. In addition, one of the five panelists was outside the "natural universe of suntan tonality" range and another panelist had a less developed tan that was on the borderline of the range. Overall, the tonality of the sunless tan generated by K was less "natural" since three out of five panelists were outside the "natural universe of suntan tonality" range. The majority of panelists preferred the sunless tanning experience associated with X. Their subjective perceptions were: X produces a more natural, more even, and longer-lasting tan that was initially less intense versus K, which generated slightly higher ΔE *ab values in 24 hours, but faded away faster after 48 hours.

An HPLC method for the analysis of DHA levels in sunless tanning formulations was established and successfully utilized to confirm DHA concentrations in the test articles. The sunless tanner market continues to grow rapidly, and our findings can help to develop and evaluate new products with superior performance to fulfill consumers' expectations.

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