SPF Pigment Dispersion Assay

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Tradename: AC Pina Colloida

Code: 12053

CAS #: 7732-18-5 & 68917-26-0 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 10581

Lot #: N230829C

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Drumwright*
Principal Investigator: *Hannah Duckett*

Test Performed:
SPF Pigment Dispersion Assay

Introduction

Mineral sunscreens containing zinc oxide are known to leave a chalky or bluish finish on the skin which is undesirable to consumers. Pigment size and dispersion play a key role in the appearance of a product on the skin. An evenly dispersed product contains small pigments and appears more natural on the skin. Reducing particle size and agglomeration increases pigment transparency resulting in a more desirable finish on the skin. Therefore, pigment dispersion in sunscreen formulations is crucial to product appearance and consumer perception.

Accordingly, an SPF Pigment Dispersion Assay was conducted to assess the ability of **AC Pina Colloida** to increase the dispersion properties of zinc oxide in sunscreen formulations with and without the presence of a standard dispersing agent Polyhydroxystearic Acid (PHS).

Assay Principle

Zinc oxide pigment dispersion was analyzed using microscopy paired with image analysis. Both hard pigment agglomerations (particles that may not be broken during dispersion) and soft agglomerates (particles that are easily disturbed during dispersion) were observed. Total area of pigment particles was calculated for each condition to assess dispersion success. A top performing product will consist of small pigment particles that do not agglomerate together and will stay well dispersed over time which will be demonstrated by a lower average pigment area.

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Materials

A. **Equipment:** Light Microscope; Silverson High Shear Mixer; Canon EOS Rebel T3 Digital Camera
B. **Reagents:** Sunscreen Base (Table 1)
C. **Software:** ImageJ Analysis Software (National Institutes of Health); Excel Analysis ToolPak (Microsoft)
D. **Other:** Microscope slides

Table 1. Sunscreen base ingredient list (*not all batches contained this ingredient)

INCI Name
Caprylic/Capric Triglyceride
Polyglyceryl-3 Polyricineoleate
Cera alba
Garcinia Indica Seed Butter
Polyhydroxystearic Acid (PHS)*
Hydrogenated Polyisobutene
Zinc Oxide & Triethoxycaprylylsilane
Water
Sodium Chloride
Lactobacillus Ferment

Methods

The ability of 5.0% **AC Pina Colloida** to disperse the pigment zinc oxide (20% in formula) was evaluated. All formulas were produced according to the specific guidelines below to ensure pigment dispersion was not impacted by the manufacturing process.

Emulsions were formulated at 75°C and ingredients were added in four different phases using the same mix times. After cooling to room temperature, emulsions were tested immediately and again 24 hours after formulation. Emulsions were retested after 30 days to determine stability of the formulas.

Four different dispersions were produced to help understand how **AC Pina Colloida** impacted sunscreen pigment dispersion both with and without the additional industry standard dispersing agent PHS:

Table 2. Descriptions of the conditions and treatments for each skin test site

Condition	PHS added?	Description
Base	No	Base Sunscreen
Base + PHS	Yes	Base Sunscreen with added PHS
5.0% **AC Pina Colloida**	No	5.0% **AC Pina Colloida** in Base Sunscreen
5.0% **AC Pina Colloida** + PHS	Yes	5.0% **AC Pina Colloida** in Base Sunscreen with added PHS

30 mg of each dispersion was applied to a glass slide and examined under a light microscope utilizing a 40x objective. Five randomly chosen regions of each slide were evaluated to verify homogeneity of each dispersion. Microscopy images were taken of each region with a digital camera and analyzed with ImageJ Analysis Software. Pigment agglomerates were identified based on light intensity relative to the rest of the emulsion and the border of each agglomeration was determined. The total agglomeration area for each image was determined and expressed in square millimeters (mm^2) by dividing the total area (exported in pixels) by a conversion factor of a given number of pixels per millimeter. An average

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total area was calculated from the five images for each condition. Three separate experiments were performed for each condition and the average of all three experiments is displayed. Data was analyzed using a t-tests with statistical significance accepted at $p \leq 0.05$.

This method observes both soft and hard agglomerates. The average total area is useful because it demonstrates the total amount of visible agglomerates present in each image. Ideally a well dispersed product will have few to no large or visible agglomerates and will therefore have a lower average total area.

Results

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated. **AC Pina Colloida** at a concentration of 5.0% was able to improve pigment dispersion in a sunscreen base.

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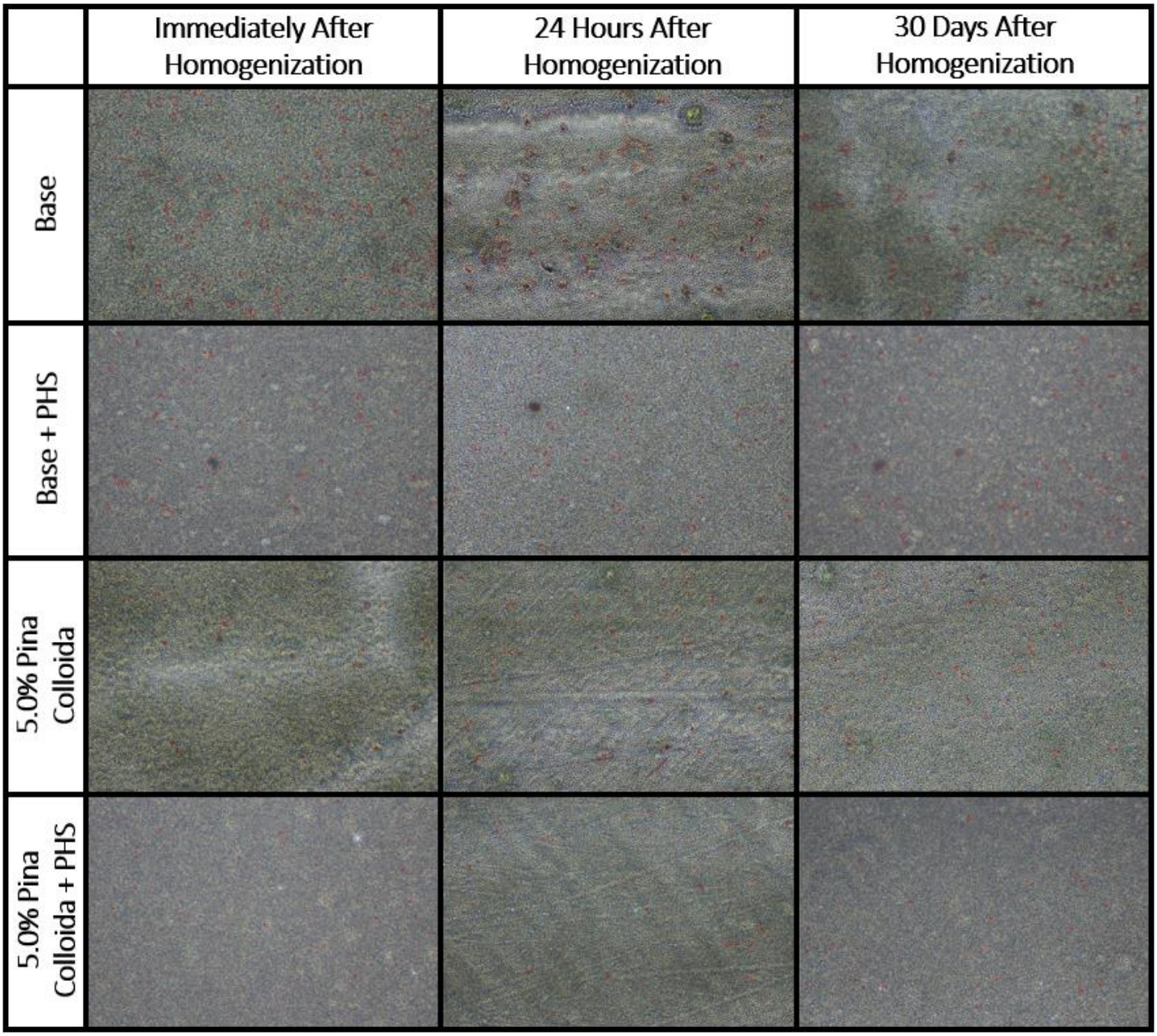

Image 1. Images of Pigment Dispersions Over Time; Agglomerates are indicated by red circles

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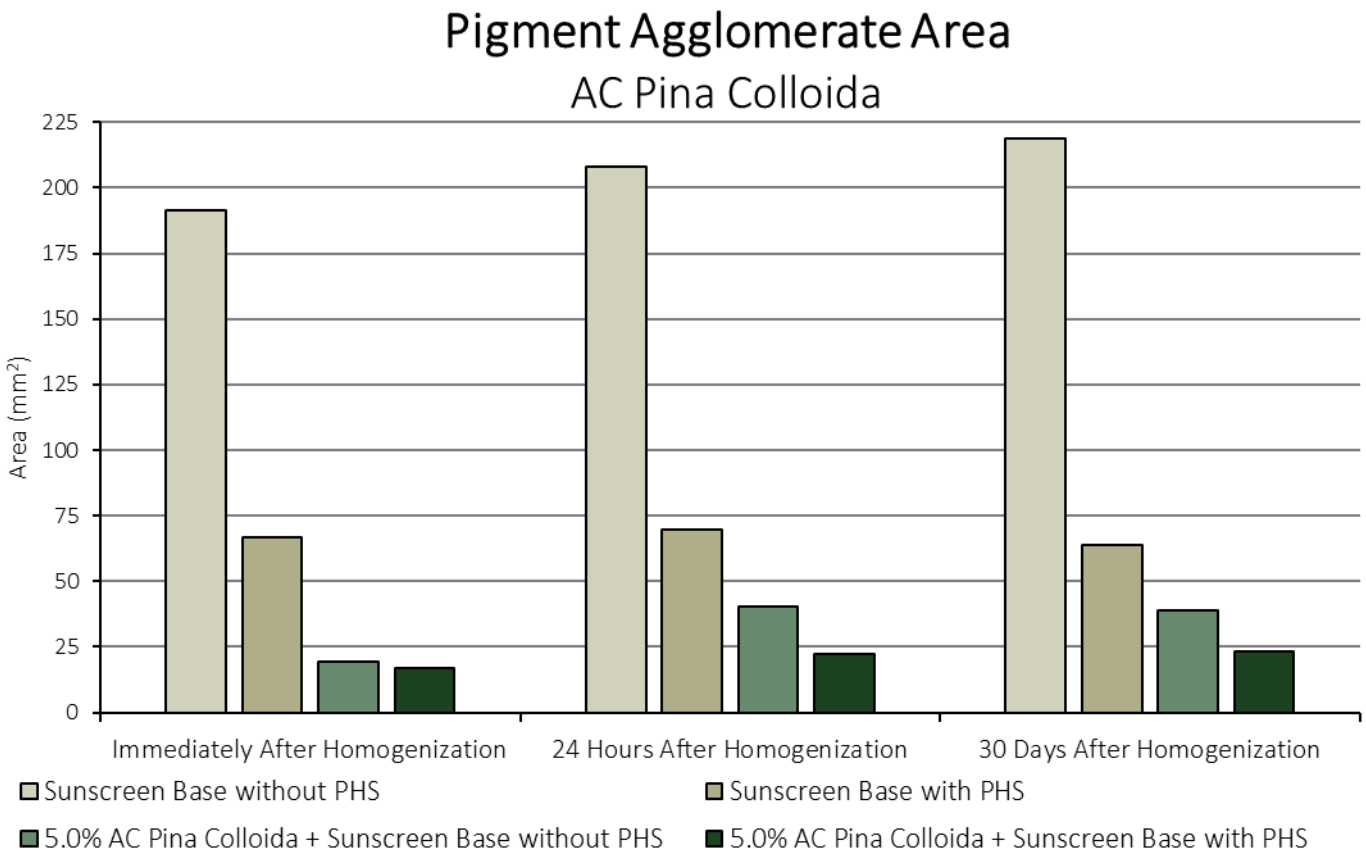

Figure 1. Area of Pigment Agglomerates (mm^2) Over Time

Table 3. Changes in Pigment Agglomeration Area between Conditions Over Time. Asterisks (*) indicate significance with a p-value < 0.05.

Percent Change (%)	Immediately After Homogenization	24 Hours After Homogenization	30 Days After Homogenization
Base vs Base + PHS	- 65*	- 67*	- 71*
Base vs 5.0% **AC Pina Colloida**	- 90*	- 81*	- 82*
Base + PHS vs 5.0% **AC Pina Colloida** + PHS	- 75*	- 68*	- 63*
5.0% **AC Pina Colloida** vs 5.0% **AC Pina Colloida** + PHS	- 14	- 45*	- 40*

Discussion

The data obtained from this study indicates **AC Pina Colloida** improves zinc oxide pigment dispersion in sunscreen formulations.

The dispersing agent PHS exhibited significant differences in pigment size within the Base conditions across all time points (Image 1, Figure 1, Table 3). Immediately, 24 hours, and 30 days after homogenization, PHS significantly reduced pigment

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agglomeration area by 65%, 67%, and 71%, respectively (Figure 1, Table 3). These results indicate PHS improves dispersion of zinc oxide in SPF formulations.

Similarly, 5.0% **AC Pina Colloida** exerted significant differences in pigment size compared to the Base at all time points (Image 1, Figure 1, Table 3). Immediately, 24 hours, and 30 days after homogenization, 5.0% **AC Pina Colloida** significantly reduced pigment agglomeration area by 90%, 81%, and 82% compared to the Base, respectively (Figure 1, Table 3). These results indicate 5.0% **AC Pina Colloida** improved dispersibility of zinc oxide in SPF formulations, to a greater degree than PHS.

In the presence of PHS, 5.0% **AC Pina Colloida** + PHS exhibited significant differences in pigment size compared to the Base + PHS across at time points (Image 1, Figure 1, Table 3). Immediately, 24 hours, and 30 days after homogenization, 5.0% **AC Pina Colloida** + PHS significantly reduced pigment agglomeration area by 75%, 68%, and 73% compared to the Base + PHS, respectively (Figure 1, Table 3). These results indicate in the presence of PHS, 5.0% **AC Pina Colloida** provides improved dispersibility of zinc oxide in SPF formulations, to a greater degree than PHS alone.

Although both formulations containing 5.0% **AC Pina Colloida** performed the best in reducing pigment size, the addition of PHS was superior. Immediately, 24 hours, and 30 days after homogenization, 5.0% **AC Pina Colloida** + PHS reduced pigment agglomeration area by 14%, 45%, and 40% compared to 5.0% **AC Pina Colloida**, respectively, however the effect of PHS was only significant 24 hours and 30 days after homogenization (Figure 1, Table 3). These results indicate 5.0% **AC Pina Colloida** in the presence of PHS provides improved dispersion as well as long-term stability to zinc oxide in SPF formulations.

Formulations without PHS tended to exhibit increased pigment areas over time indicating reduced stability. Both the addition of PHS and of **AC Pina Colloida** helped prevent increased pigment areas over time. When used in conjunction, **AC Pina Colloida** + PHS had the best stability over time compared to the other formulations.

In summary, these results indicate **AC Pina Colloida** provides improved zinc oxide pigment dispersion by reducing individual pigment size when added to SPF cosmetic products at normal use concentrations. Additionally, in the absence and presence of PHS, **AC Pina Colloida** provided superior dispersion of zinc oxide than the base counterpart. Collectively, **AC Pina Colloida** demonstrates effective zinc oxide pigment dispersing properties which improves overall skin coverage and product appearance.