# 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 #:** 10582

**Lot #:** N230829C

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

**Test Performed:**
Color Pigment Dispersion Assay

## Introduction

The role of pigment dispersion in color cosmetics is crucial to product function and appearance. A product that disperses pigments evenly will provide better overall coverage and can improve the product's appearance on the skin. A well dispersed product will consist of small pigment particles that are not agglomerated together and will stay dispersed over time. Improving pigment dispersion can also positively impact color intensity of a product as well, such as in foundations, blushes, or eyeshadows.

Accordingly, a Color Pigment Dispersion Assay was conducted to assess the ability of **AC Pina Colloida** to increase the dispersion of individual and combined color pigments in cosmetic products.

## Assay Principle

A multiparameter approach was taken to assess pigment dispersion in cosmetic formulations. Individual pigment dispersions provide insight on particle size and count, pigment sedimentation over time, and color intensity, while a blend of pigments offers understanding of how pigments will disperse as part of a cosmetic formula. A well dispersed pigment will consist of small particles close to the primary particle size which in turn increase particle count. Decreased particle size typically translates to a more stable dispersion over time as there is less sedimentation of large agglomerates. Smaller pigment sizes may also boost color intensity via an increased surface area exposed to light. The final blend of pigments further demonstrates decreased pigments sizes and provides more realistic formulation conditions.

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## Materials

A. **Equipment:** Light Microscope; ImageJ (NIH) Software; Silverson High Shear Mixer; Canon EOZ Rebel T3 Digital Camera; Certified Elcometer 2020 Fineness of Grind Gauge 0 – 50 µm
B. **Reagents:** Propanediol; Unipure White LC 987 BA; Unipure Red LC 381 BA; Unipure Yellow LC 182 BA; Unipure Black LC 989 BA
C. **Software:** Excel Analysis ToolPak (Microsoft)
D. **Other:** Test tubes, Filter Paper, Pipets

## Methods

The ability of 5.0% **AC Pina Colloida** to disperse individual pigments was evaluated against three different criteria, whereas dispersion with a blend of color pigments was assessed by one metric. Each assessment is explained in detail below. The amount of pigment utilized for individual and blended experiments replicates pigment concentrations in foundation formulas (Table 1). For individual and blended experiments, pigments were suspended in Propanediol or Propanediol with 5.0% **AC Pina Colloida** and homogenized for five minutes. During homogenization, samples were taken at 30 seconds, 1 minute, 3 minutes, and 5 minutes to evaluate dispersion progress.

T-tests were performed to evaluate the statistical significance between Propanediol and Propanediol with 5.0% **AC Pina Colloida**. Values marked with an asterisk (*) indicate significance, which was accepted at $p < 0.05$.

### *A. Individual Pigment Particle Size Analysis*

During the five-minute homogenization, samples were taken at 30 seconds, one minute, three minutes, and five minutes. Immediately following homogenization, one drop from each time point was placed between two microscope slides and examined under a light microscope with a 40x objective. Both conditions were evaluated at each time point and five randomly chosen regions of each slide were selected for analysis. Particle size and count were quantified from microscopy images using ImageJ and averages of the five regions were recorded. The inverse relationship between count and particle size indicates pigment dispersion over time (i.e., particle size decreases and particle count increases).

### *B. Individual Pigment Color Intensity Analysis*

During the five-minute homogenization, samples were taken at 30 seconds, one minute, three minutes, and five minutes. Immediately following homogenization, three drops from each time point were placed onto filter paper and allowed to dry for five minutes. Digital images were captured, and ImageJ was utilized to determine color intensity. Experiments and analyses were performed in triplicate and an average was recorded. Enhanced red and yellow pigment dispersions are observed as having brighter intensities, while black pigment dispersions will shift towards darker intensities.

### *C. Individual Pigment Sedimentation Assessment*

After the five-minute homogenization, 8 mL of each pigment solution was added to a glass test tube, sealed, and incubated at room temperature for one day. Images of each solution were recorded immediately following homogenization and after the 24-hour incubation. A well dispersed pigment will remain homogenous over time with little to no precipitation or color change.

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### *D. Pigment Blend Particle Agglomeration Test*

After the four pigments were homogenized for five minutes, particle agglomeration and fineness were evaluated using a certified grind gauge. Approximately 2 mL of each condition was added to the deep end of the dual wells and drawn down with a flat edge at a constant speed and pressure. The fineness rating (µm) was determined where five particles/streaks became visible on the gauge as this is the point where oversized particles start to appear in high density. All test materials were tested in triplicate and an average pigment size was determined. Well dispersed pigments will have lower ratings demonstrating reduced particle agglomeration and increased particle fineness.

**Table 1.** Pigment Compositional Breakdown for Individual and Blended Experiments

| Tradename | Individual Pigment Experiment Concentrations (%) | Pigment Blend Experiment Concentration (%) |
|---|---|---|
| Propanediol | Dependent on Pigment Concentration | 55.17 |
| Unipure White LC 987 BA | Not Tested Individually | 41.38 |
| Unipure Red LC 381 BA | 1.00 | 1.03 |
| Unipure Yellow LC 182 BA | 2.00 | 1.72 |
| Unipure Black LC 989 BA | 0.50 | 0.69 |

## Results

The data obtained from this study met criteria for a valid assay and the Propanediol control performed as anticipated. Compared to Propanediol, 5.0% **AC Pina Colloida** improved individual pigment dispersion regarding size and count, color intensity, sedimentation, and particle size with a blend of pigments. Values marked with an asterisk (*) indicated significance (<0.05).

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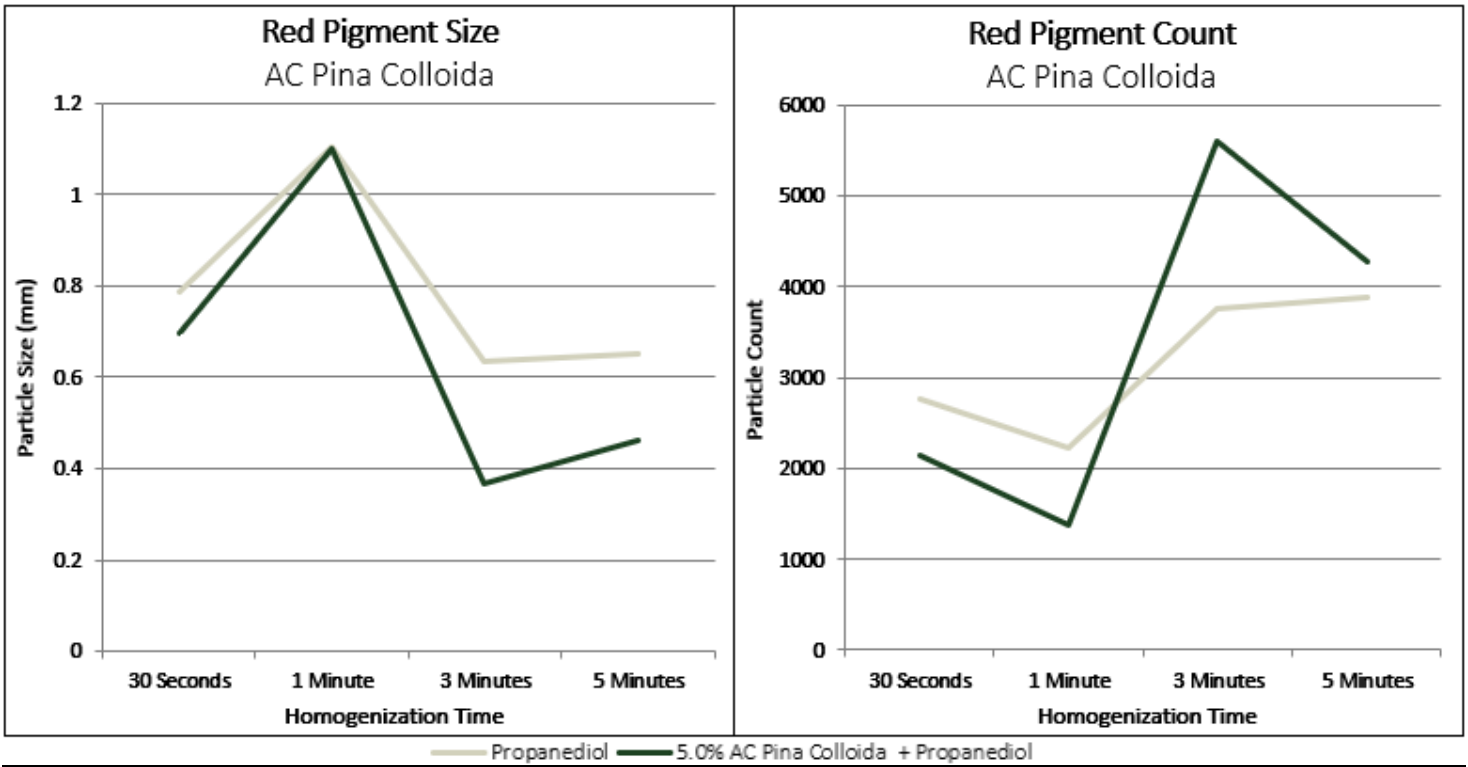
*A. Individual Pigment Particle Size Analysis*




| Percent Change (%) | | 30 Seconds vs 1 Minute | 30 Seconds vs 3 Minutes | 30 Seconds vs 5 Minutes |
|---|---|---|---|---|
| Size | Propanediol | 41 | -19 | -17 |
| | 5.0% AC Pina Colloida | 58 | -47* | -34* |
| Count | Propanediol | -20 | 36 | 41 |
| | 5.0% AC Pina Colloida | -36 | 163* | 100* |

| Percent Difference (%) | | 30 Seconds | 1 Minute | 3 Minutes | 5 Minutes |
|---|---|---|---|---|---|
| Size | Propanediol vs 5.0% AC Pina Colloida | 12 | 0 | 53* | 34* |
| Count | Propanediol vs 5.0% AC Pina Colloida | 26 | 48 | 40* | 9* |

**Figure 1.** Microscopy Particle Analysis for 1.0% Unipure Red LC 381 BA in Propanediol and 5.0% AC Pina Colloida Over Time

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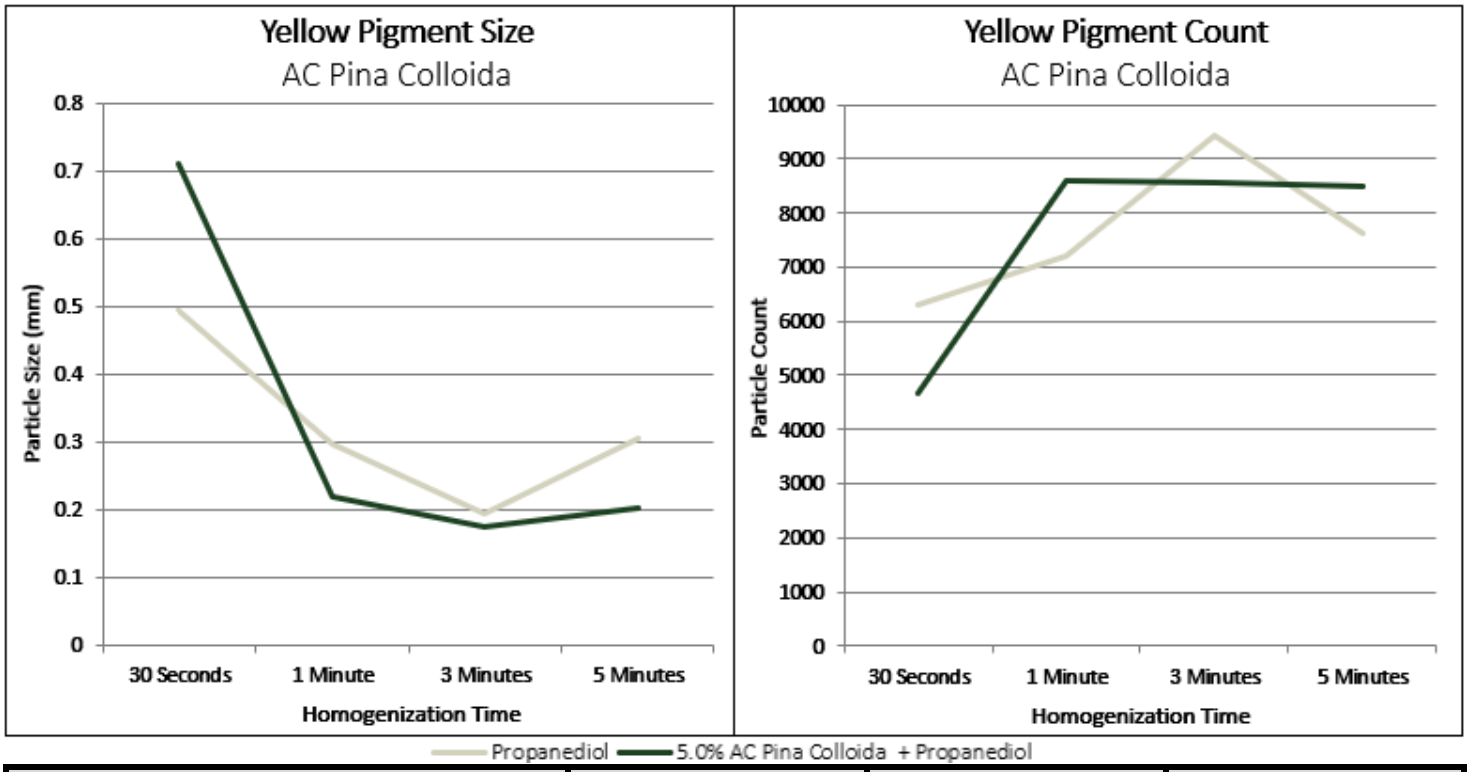


| Percent Change (%) | | 30 Seconds vs 1 Minute | 30 Seconds vs 3 Minutes | 30 Seconds vs 5 Minutes |
|---|---|---|---|---|
| Size | Propanediol | -40 | -61* | -39 |
| | 5.0% AC Pina Colloida | -69* | -75* | -72* |
| Count | Propanediol | 14 | 50* | 21 |
| | 5.0% AC Pina Colloida | 84* | 83* | 81* |

| Percent Difference (%) | | 30 Seconds | 1 Minute | 3 Minutes | 5 Minutes |
|---|---|---|---|---|---|
| Size | Propanediol vs 5.0% AC Pina Colloida | 36 | 30 | 10 | 40* |
| Count | Propanediol vs 5.0% AC Pina Colloida | 30 | 18 | 10 | 11* |

**Figure 2.** Microscopy Particle Analysis for 2.0% Unipure Yellow LC 182 BA in Propanediol and 5.0% **AC Pina Colloida** Over Time

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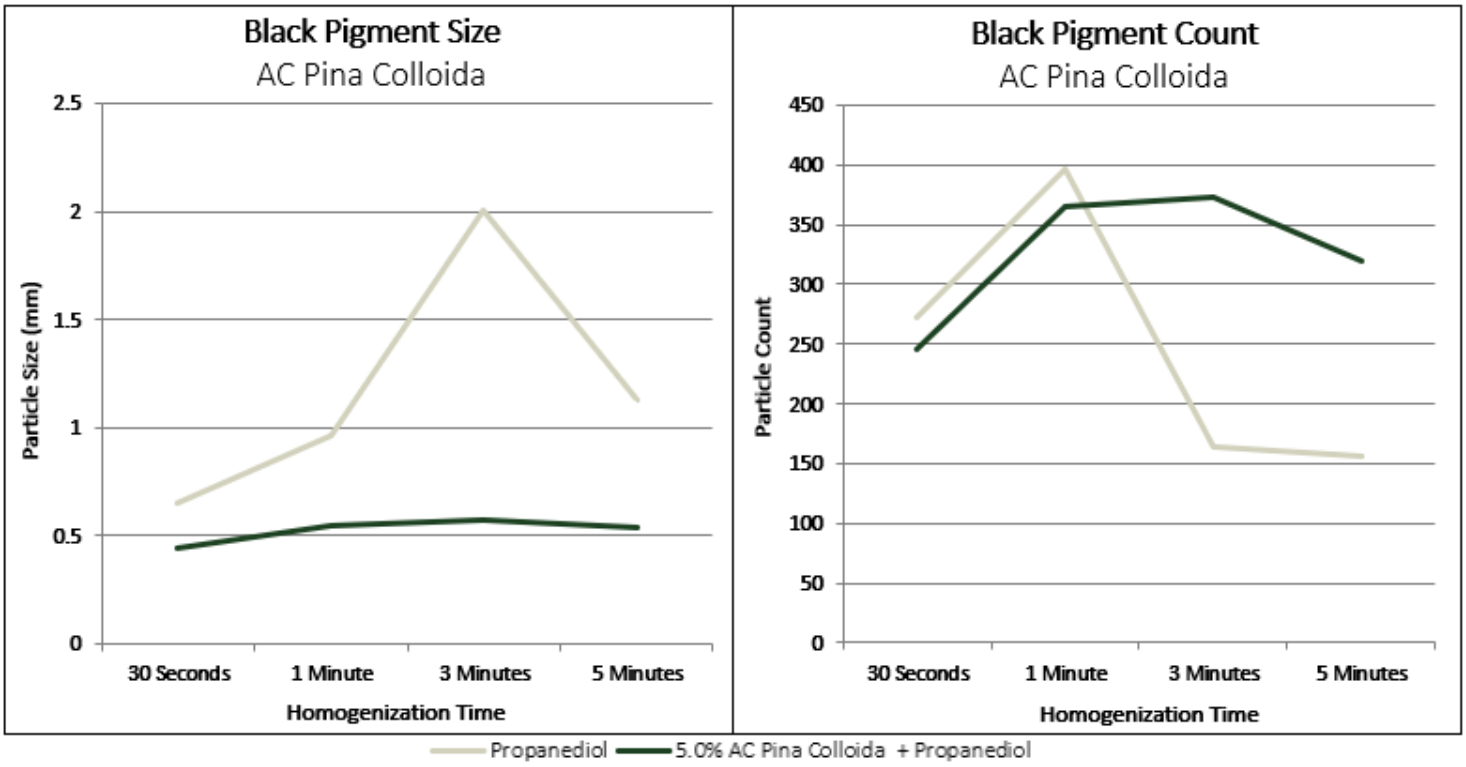


| Percent Change (%) | | 30 Seconds vs 1 Minute | 30 Seconds vs 3 Minutes | 30 Seconds vs 5 Minutes |
|---|---|---|---|---|
| Size | Propanediol | 48 | 207* | 72 |
| | 5.0% **AC Pina Colloida** | 24 | 30 | 22 |
| Count | Propanediol | 45* | -40 | -43* |
| | 5.0% **AC Pina Colloida** | 48* | 51* | 30* |

| Percent Difference (%) | | 30 Seconds | 1 Minute | 3 Minutes | 5 Minutes |
|---|---|---|---|---|---|
| Size | Propanediol vs 5.0% **AC Pina Colloida** | 40 | 56 | 111* | 71* |
| Count | Propanediol vs 5.0% **AC Pina Colloida** | 10 | 8 | 78* | 69* |

**Figure 3.** Microscopy Particle Analysis for 0.5% Unipure Black LC 989 BA in Propanediol and 5.0% **AC Pina Colloida** Over Time

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*B. Individual Pigment Color Intensity Analysis*

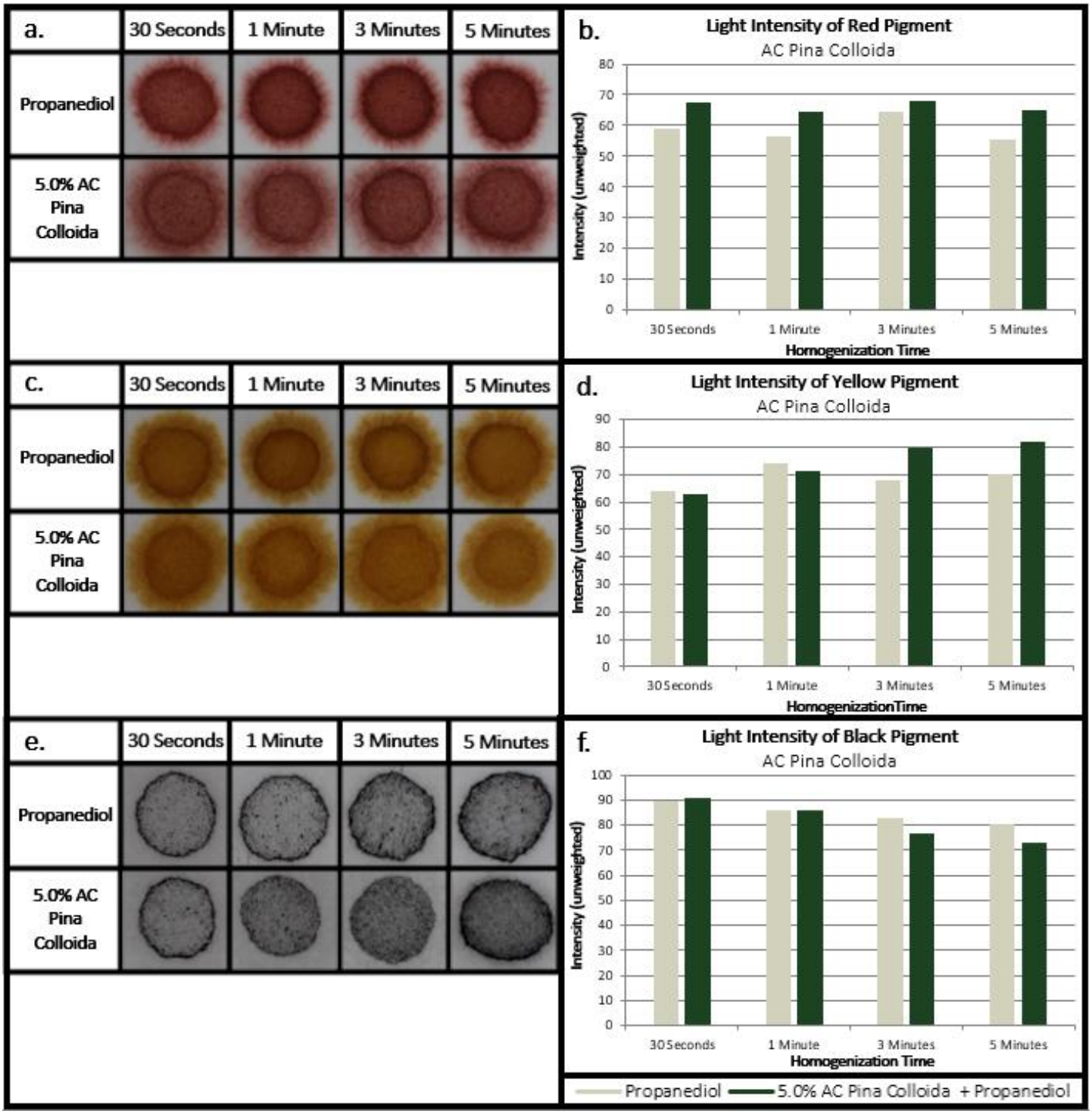


**Figure 4.** Color Intensity Analysis for 1.0% Unipure Red LC 381 BA (a-b), 2.0% Unipure Yellow LC 182 BA (c-d), & 0.5% Unipure Black LC 989 BA (e-f) in Propanediol and 5.0% **AC Pina Colloida** Over Time

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**Table 2.** P-values for Color Intensity Differences between Propanediol and 5.0% **AC Pina Colloida** for each Pigment Over Time

| Percent Difference (%) | 30 Seconds | 1 Minute | 3 Minutes | 5 Minutes |
|---|---|---|---|---|
| 1.0% Unipure Red LC 381 BA | 0.015* | 0.045* | 0.781 | 0.042* |
| 2.0% Unipure Yellow LC 182 BA | 0.174 | 0.126 | 0.003* | 0.021* |
| 0.5% Unipure Black LC 989 BA | 0.747 | 0.993 | 0.324 | 0.044* |

*C. Individual Pigment Sedimentation Assessment*

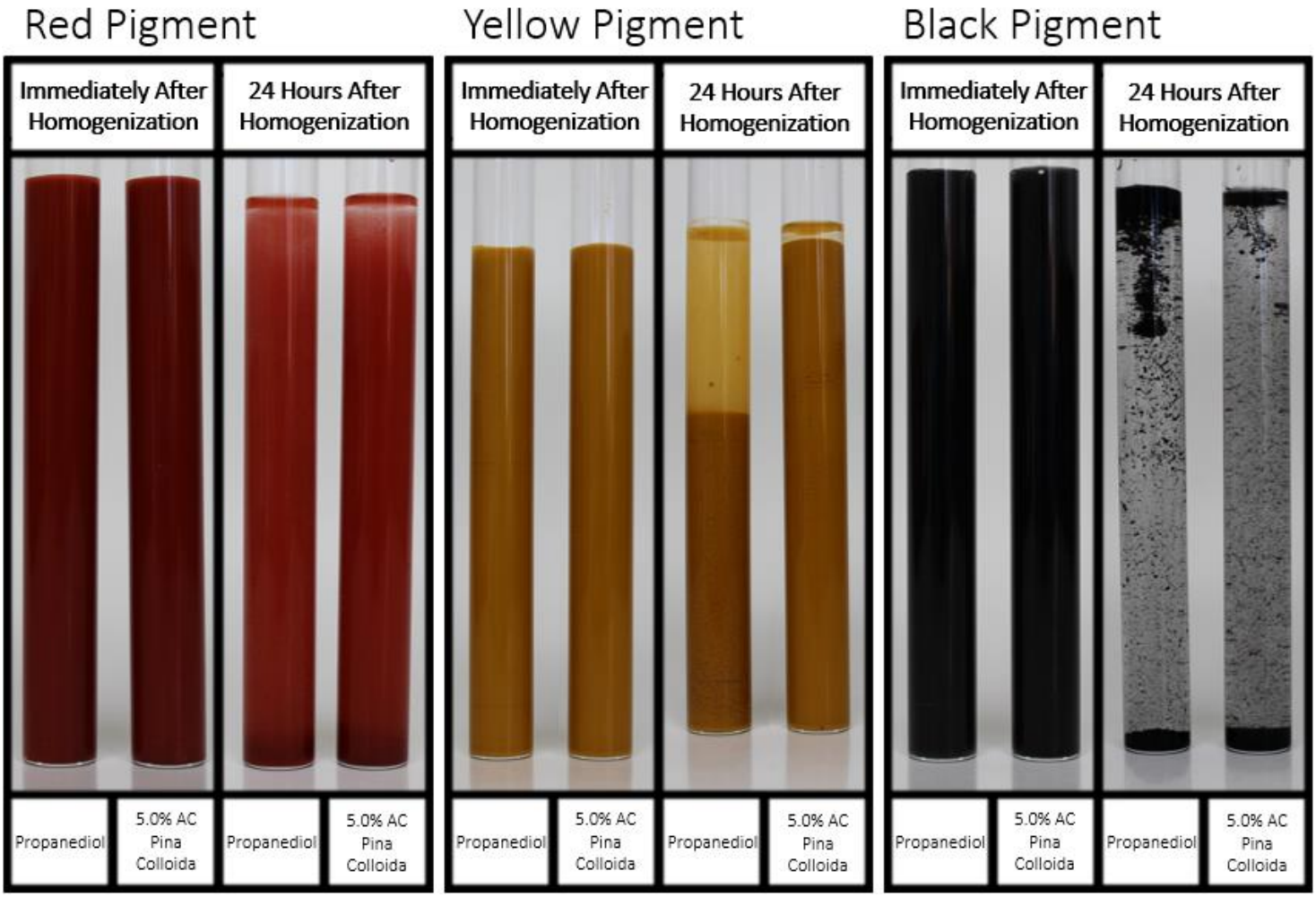


**Image 1.** Pigment Sedimentation in Propanediol and 5.0% **AC Pina Colloida** Immediately and 24 hours After Homogenization

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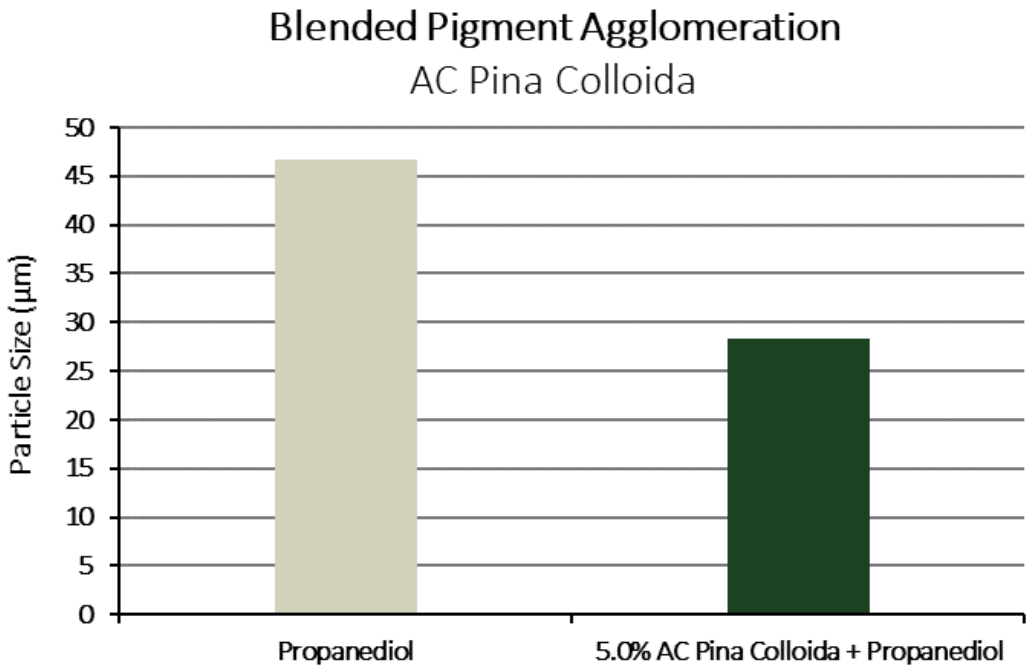
*D. Pigment Blend Particle Agglomeration Test*




| | Percent Change (%) |
|---|---|
| Propanediol vs 5.0% **AC Pina Colloida** | -39* |

**Figure 5.** Grind Guage Particle Size (µm) of a Four-Pigment Blend in Propanediol and 5.0% **AC Pina Colloida** Immediately After Homogenization. Well dispersed pigments will have lower ratings demonstrating reduced particle agglomeration and increased particle fineness.

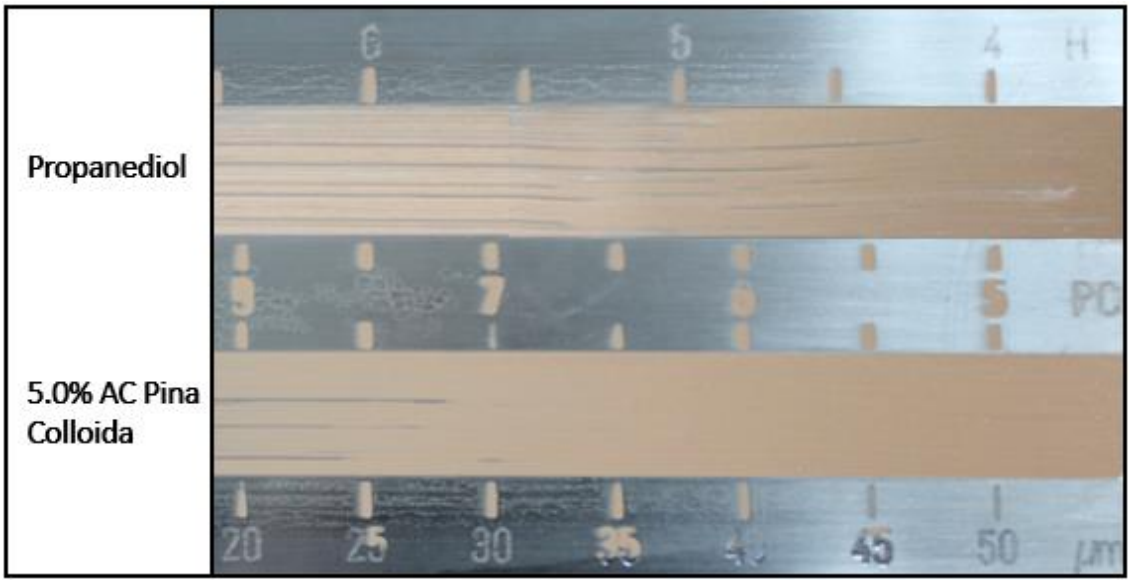



**Image 2.** Grind Gauge Image of Each Pigment Dispersion

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## Discussion

The results from this study indicate 5.0% **AC Pina Colloida** improves pigment dispersion in color cosmetics. **AC Pina Colloida** effectively dispersed individual pigments across three different criteria compared to Propanediol and reduced agglomeration with a blend of pigments better than Propanediol against one metric.

### *A. Individual Pigment Particle Size Analysis*

The first individual pigment assessment examined the effect of 5.0% **AC Pina Colloida** on pigment particle size. After one, three, and five minutes of homogenization, 5.0% **AC Pina Colloida** reduced the particle size of all three color pigments compared to Propanediol (Figures 1-3). Specifically, after five minutes of homogenization, Propanediol decreased the red and yellow pigment sizes by 17% and 39%, respectively, whereas the black pigment demonstrated a 72% increase in size (Figures 1-3). Conversely, after the same amount of time, 5.0% **AC Pina Colloida** produced decreases of 34% and 72% in red and yellow pigment size, respectively, but only produced an increase of 22% for the black pigment (Figures 1-3). As expected, particle count exhibited an inverse relationship to particle size for all the pigment homogenizations after one minute (Figures 1-3). This is indicative of large pigment agglomerates being reduced to sizes closer to primary pigment size. These results indicate pigments in the presence of 5.0% **AC Pina Colloida** exhibit smaller pigment sizes and increased particle count illustrating enhanced pigment dispersion.

### *B. Individual Pigment Color Intensity Analysis*

The second individual pigment assessment examined the effect of 5.0% **AC Pina Colloida** on pigment color intensity. After five minutes of homogenization, 5.0% **AC Pina Colloida** significantly enhanced red and yellow pigment color intensity by 13% and 17% compared to Propanediol (Figure 4, Table 2). Additionally, 5.0% **AC Pina Colloida** significantly darkened the black pigment color intensity by 9%. Together, these results demonstrate **AC Pina Colloida** augments the color intensity of pigment dispersions in color cosmetics.

### *C. Individual Pigment Sedimentation Assessment*

The third individual pigment assessment examined the effect of 5.0% **AC Pina Colloida** on pigment sedimentation and stability. Visually, 5.0% **AC Pina Colloida** reduced sedimentation and improved stability with red, yellow, and black pigment dispersions 24 hours after homogenization (Image 1). These data indicate **AC Pina Colloida** maintains a uniform pigment dispersion with little to no precipitation or color change following homogenization.

### *D. Pigment Blend Particle Agglomeration Test*

The pigment blend assessment examined the effect of 5.0% **AC Pina Colloida** on pigment agglomeration and fineness. Well dispersed pigments will have lower ratings demonstrating reduced particle agglomeration and increased particle fineness. After a five-minute homogenization, 5.0% **AC Pina Colloida** reduced particle agglomeration in the four-pigment blend by 39% compared to Propanediol (Figure 5, Image 2). These results indicate **AC Pina Colloida** enhances blended pigment dispersion by reducing particle agglomeration and increasing particle fineness.

In summary, these results indicate **AC Pina Colloida** provides homogenous color dispersions by reducing individual pigment size, improving pigment color intensity, reducing pigment sedimentation, and reducing blended pigment agglomeration when added to color cosmetic products at normal use concentrations. Collectively, **AC Pina Colloida** demonstrates effective pigment dispersing properties which improves overall skin coverage and product appearance.