BSA IS NOT ALWAYS GREENER ON THE OTHER SIDE

DOES COUNTRY OF ORIGIN AFFECT BSA COLOR?

INTRODUCTION

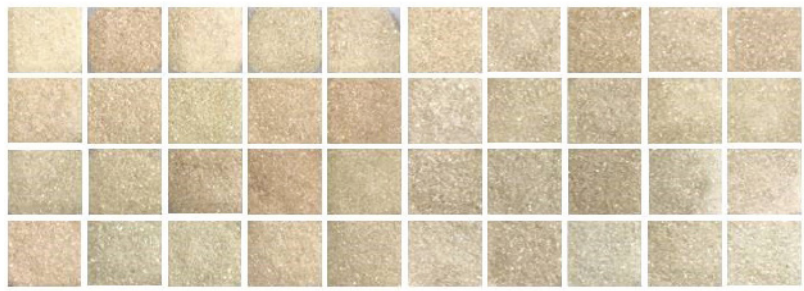
Bovine Serum Albumin (BSA) is a 66kDa protein that regulates osmotic pressure in blood. BSA is a commonly used protein in human diagnostics, vaccine production, and recombinant protein therapeutics manufacturing. Due to the critical nature of its applications, BSA products need to be characterized and consistent lot-to-lot. Due to the ease of observation, color can be used as a measure of product consistency. How color relates to product function is often unknown but could be determined to evaluate its use as a relevant measure of product consistency for a specific application. The challenges with using general descriptions of color as a metric are the same challenges color science tries to address—inadequate communication, variable illuminants, and variable observers. By combining the objective metrics of color science with a systematic analysis of lots and grades it is possible to understand lot-to-lot variability and product similarity in a quantifiable way. To this end Proliant Health & Biologicals (PHB) has undertaken a study to better understand the range and variability of our BSA product lines using colorimetry.

The perception, interpretation, and communication of colors seems straightforward and simple, yet it is quite complex and challenging. Two people can perceive the color of the same object differently even when the observation conditions are exactly the same. While one person may see a ball and describe the color as red another person may describe the same ball as orange. The two people can view the exact same color object under the exact same conditions and still interpret the color differently as their perceptions of color are different. In this example, the communication of the color is insufficient. An entire discipline on the science of color evolved to improve the communication about color while controlling for differences in description, observation, and perception.

The science of color quantifies color using standardized measurements so the exact same paint can be matched when restoring a car, and movies can be viewed using the same colors as when they were produced.

Figure 1: *Images of different lots of BSA powders produced in the USA or New Zealand taken under non-controlled conditions.*

PHB UNITED STATES POWDERS



PHB NEW ZEALAND POWDERS

The goal is to quantify colors using a scale that matches human perception so we can better communicate about color with others. We can use these scales to understand variations in color and determine the range of colors that are perceived to be the same or different. Ultimately visual perception of color is the most important measurement. But a good scale allows for a more objective analysis and communication of color.

The CIE L*a*b* scale of color was developed by the International Commission on Illumination in 1976. It was named L*a*b* to distinguish it from the previously existing Hunter Lab scale named L, a, b which was created in 1948. The CIE L*a*b* is a three-dimensional color space designed to match human perception of color space, meaning the distance between two colors in L*a*b* space is directly related to how similar or different they look with the axis scaled to human perception. Cone cells in the eye respond to red, green, and blue light but the nerve impulses sent to the visual cortex are signaled based on lightness and opponent colors such as red-green and yellow-blue (Lindon, Tranter, & Koppenaal, 2010). This means a person cannot perceive a color as being simultaneously red and green or yellow and blue. The CIE tristimulus variables L*, a*, and b* mirror the physiological nerve impulses. L* is a measure of lightness, ranging from 0 (total black, complete absorption of light) to 100 (absolute white), a* is a measure of red-green (positive to negative), and b* is a measure of yellow-blue (positive to negative) Figure 2. CIE L*a*b* measurements require three criteria for making a measurement: an object, an illuminant light source, and an observer. A reference illuminant accounts for how an object changes color based on the light source, such as daylight or an incandescent lamp. A standard observer reference is also required to account for how the average person sees color across the visible spectrum.

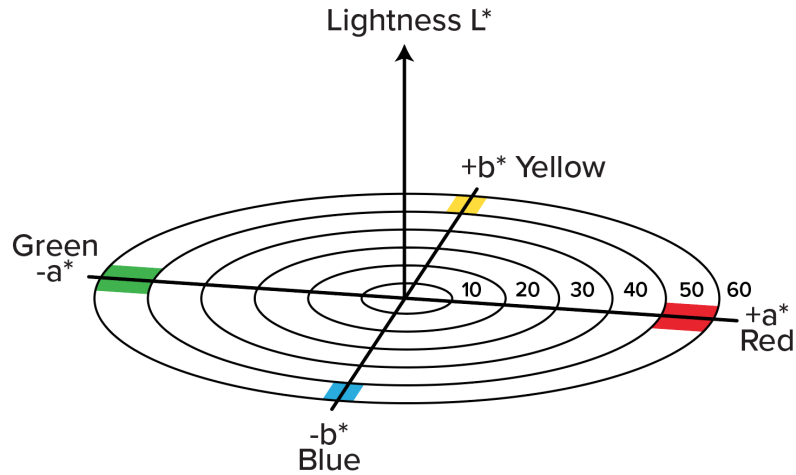


Figure 2: *CIE L*a*b* Color Space. Image taken from A Guide To Understanding Color (X-Rite, 2016).*

This white paper will present an objective look at the range of colors within Proliant's BSA products grouped by form (powder or liquid) and country of origin. CIE L*a*b* analytics will be used to quantify color variation and represent that variation as real colors as well as numbers.

MATERIALS & METHODS

MATERIALS – PHB United States (US) Standard Grade pH 7 (SKU 68100, N=28), US Reagent Grade Fatty Acid Free (SKU 68700, N =25), New Zealand (NZ) Standard Grade pH 7 (SKU 69100, N=20), NZ Reagent Grade pH 7 Fatty Acid Free (SKU69700, N=13), and NZ Precision Grade Fatty Acid Free (SKU 69750, N=18) products were sourced (Boone, IA or Feilding, NZ). Lots from different years were analyzed to reduce effects of aging on color.

METHODS – Color measurements were made using a Hunter Labs Miniscan XE Plus Colorimeter calibrated using white and black tiles on the daylight setting and a D65/10 degree reference. Lyophilized BSA powders were measured in white weigh boats, approximately 20 grams in a 3.5" by 3.5" weigh boat, with a single reading performed on each sample. BSA solutions were prepared at 20% (w/v) in deionized water. The liquid reservoir was filled with 50mL of the 20% BSA solution, and measurements were taken using the white backdrop and light blocking cup.

Data were output in Hunter Labs L, a, b color space and converted to CIE L*a*b* values (EasyRGB, 2020) and D65/10 illuminant and observer values (refX 94.811, refY 100, refZ 107.304). Translation from Hunter L, a, b to CIE L*a*b* values allowed for color analysis to be performed using MATLAB and GraphPad Prism. Color swatches were prepared using web-based data visualization software (Johnstone, 2020). Color difference value Δa* was calculated as previously described (Lindon et al., 2010; X-Rite, 2016).

RESULTS & DISCUSSION

When quantifying the color of BSA powders it is important to put the numbers in an understandable context. While comparing the numbers is important, understanding the actual colors those values represent is important to understanding the analysis. Figure 3 shows the 3D color space in CIE L*a*b* scale. A cross section of the cube was taken at the average luminosity (L* = 80) of the BSA powders. A zoomed insert then shows the small region of a* and b* space where the BSA powders' CIE L*a*b* color values range. All of the BSA powders analyzed exist in a small color space which is a fraction of the variation of greens, yellows, and browns.

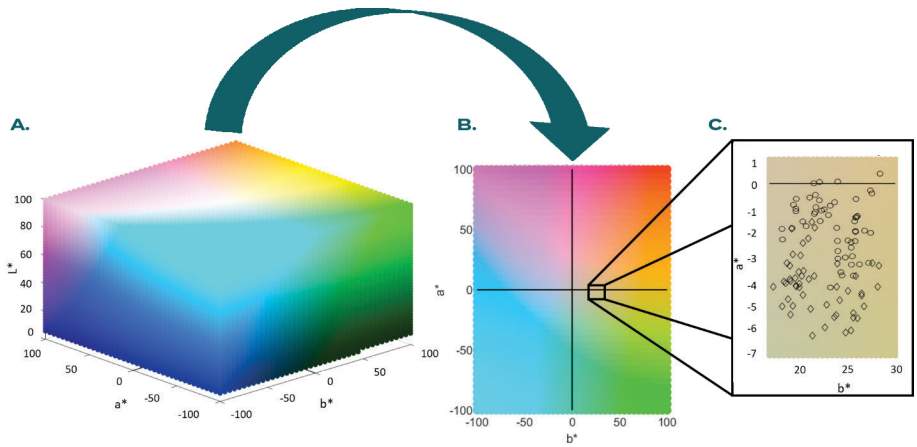


Figure 3: *Location of BSA Powders in 3D color space. A) Three-dimensional CIE L*a*b* color space sliced into a B) 2D projection at the average L*=80 value, for BSA powders and C) zoomed into to highlight the range of observed colors for BSA powders. Marker colors plotted in C) are the real L* a* b* colors for each lot. BSA lots produced in the US displayed as circles and New Zealand lots as diamonds.*

The a* data represents variation of green while the b* data shows changes in yellow. It is important to understand how changes in both values change the perceived color. Looking at the a* versus b* plot in Figure 4, the lot-to-lot color variability is apparent for both powder and solutions. The variation of a*, b*, and L* for powders is much smaller for powders than liquids, Table 1, with most of the variation occurring in the b* and L* values. Interestingly the range of green values, a*, is almost the same for the powders and liquids.

	AVERAGE CIE VALUES			
	POWDERS		SOLUTIONS	
	US	NZ	US	NZ
L*	80.4	79.0	61.4	64.7
a*	-1.7	-4.1	-3.6	-6.4
b*	23.7	22.1	50.6	50.7
N	53	51	40	50

	STANDARD DEVIATIONS			
	POWDERS		SOLUTIONS	
	US	NZ	US	NZ
L*	1.5	2.1	5.4	5.0
a*	1.1	1.1	1.3	1.2
b*	2.6	3.0	12.8	15.6
N				

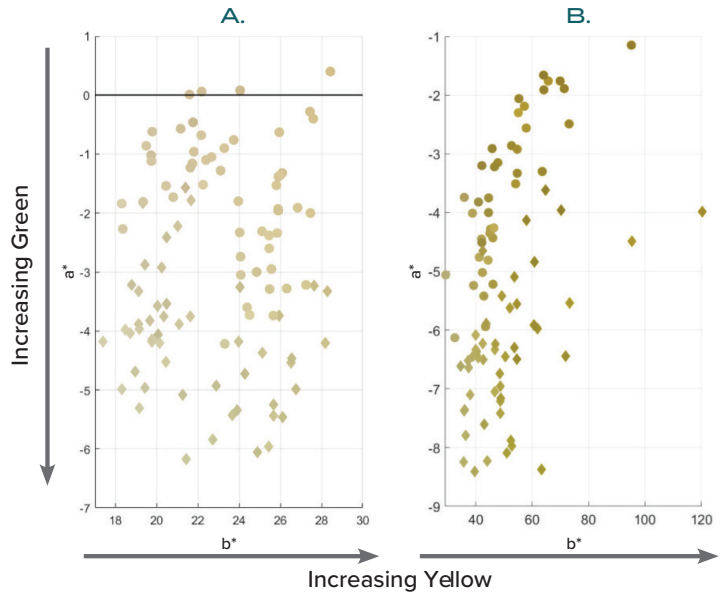
Table 1: *Average CIE L*, a*, b* values for BSA powders and 20% solutions*

When looking at the variation in color of the powders, Figure 4a, the most extreme differences (upper right and lower left) show a clear distinction between a yellow-brown color and a green-yellow color. While it is harder to distinguish the colors in the space in-between. For the solutions there is a more marked color change from upper right to lower left, Figure 4b, driven not by how red-green the solutions are, but rather by their yellow-blue and L* characteristics. The uncertainty of these lot-to-lot color variations on application performance can create unease, but quantification of color makes objective comparisons of lots possible. So, if one wants, they could determine if a correlation exists between lot-to-lot application performance and lot-to-lot color.

Looking at the values for powders, both US and NZ lots have the same range of yellow-blue b* values, 17-28. The red-green a* values from US and NZ powder lots have a similar spread, but the US data set is slightly higher (less green) than the NZ lots, with an a* range of 0.4 to -4.2 versus -1.6 to -6.2. This data points to the NZ powders being slightly greener than US powders. Yet when you look at the data points in Figure 4a, there is a clear overlap of the US and NZ powders in the a* b* scale. A simpler way of looking at the data is by comparing a box and whisker plot of a* values between lots of BSA powders from US and NZ, Figure 5. While the NZ lots of BSA powders have a lower median a*-value than the US lots, the upper two quartiles of the NZ lots overlap with the lower two quartiles of the US lots. In statistical terms, the lower two quartiles for the US lots have the same a* values as the upper two quartiles for the NZ lots.

But as with any BSA product, different grades and lots should be evaluated to determine their performance in any specific application.

Figure 4: *2D plot of a* vs b* values for lots of A) BSA powders and B) 20% BSA solutions. Markers filled with each lot's color determined by their CIE L*, a*, b* values. BSA lots produced in the US shown as circles and NZ lots shown as diamonds.*

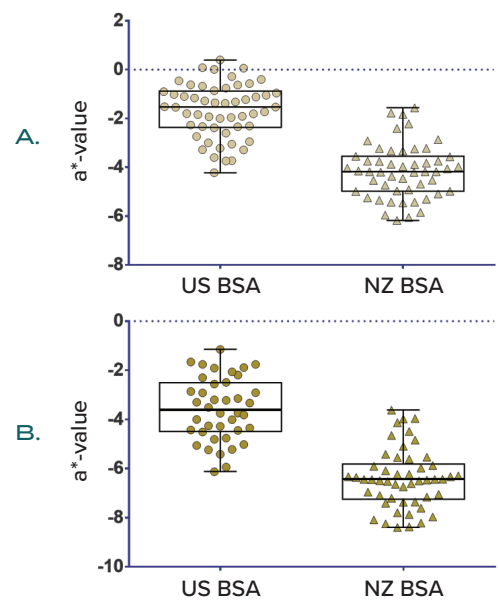


The fifty percent overlap in a values means there is significant chance any individual lot of BSA from the US could be greener than a lot from NZ, and any lot from NZ could be browner than a lot from the US. Thus, if color is an indicator of performance and both green and brown lots from the US perform well in an application, then both green or brown lots from NZ should also perform well in that application.*

Figure 5b shows BSA liquids follow a similar statistical trend as was observed for the powders. The median a* value for NZ BSA is greener than for US BSA, but the upper two quartiles of the NZ lots are the same as the lower two quartiles of the US lots. Again, this shows that one-in-four lots of US BSA are expected to result in a greener solution than any lot of NZ BSA. Additionally, different grades of BSA have been compared by origin and are shown to have the same trends of significant color overlap.

To better visualize the lot-to-lot variation in color for US and NZ powders and solutions, the L*a*b* values in Table 1 are displayed as color swatches in Figure 6. The swatches show the variation of colors that can be expected in 95% of lots produced. These swatches support the analyses in Figures 3 and 4 that the variation in colors between US and NZ products show significant overlap by number and by eye.

Figure 5: *A) Box and whisker plots of a* values for lots BSA powders and B) 20% BSA solutions. Marker colors displayed as average L*a*b* values for the group. US lots displayed as circles. NZ lots displayed as diamonds.*



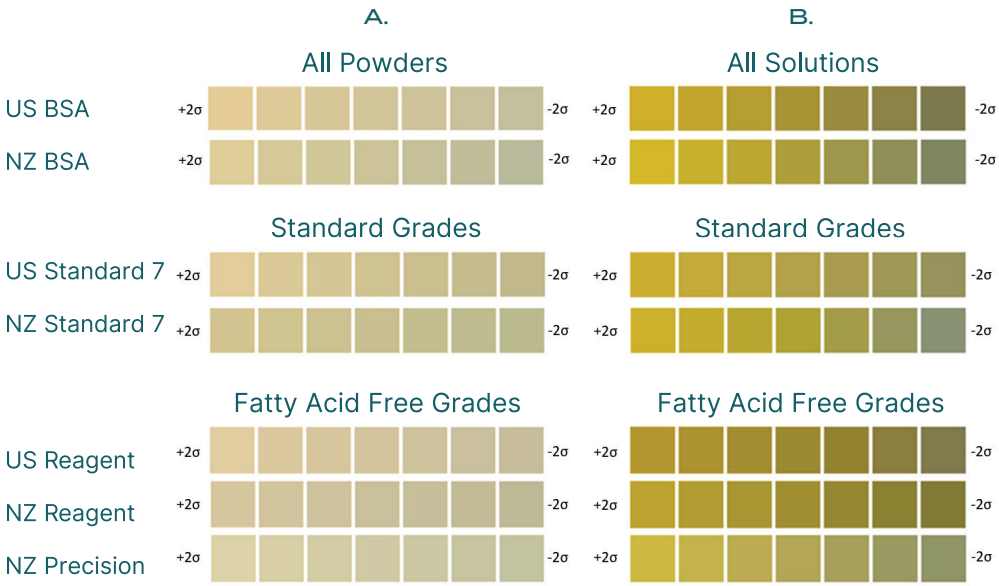


Figure 6: *Color swatches for BSA powders showing +/- two standard deviations from the average color value (L*, a*, b*) of the group. STD indicates Proliant Standard 7.0 Grades, REA are Proliant Reagent Grades, and PRE is Proliant Precision Grade. B) Color swatches for BSA solutions showing +/- two standard deviations from the average color value of the group.*

Analyzing the similarity and differences between the US and NZ powders using Δa* and ΔE values further supports the conclusions that both sets of powders have similar variabilities and significant overlap of colors. Δa* is simply the difference between the red-green a* values of a reference color and a sample color (Lindon et al., 2010; X-Rite, 2016). A Δ unit of 1.0 means that 50% of observers would perceive a color difference between the two samples (X-Rite, 2016).

Stated another way, the average US and average NZ powder are more similar in their green color than lots from the same country of origin are with each other.

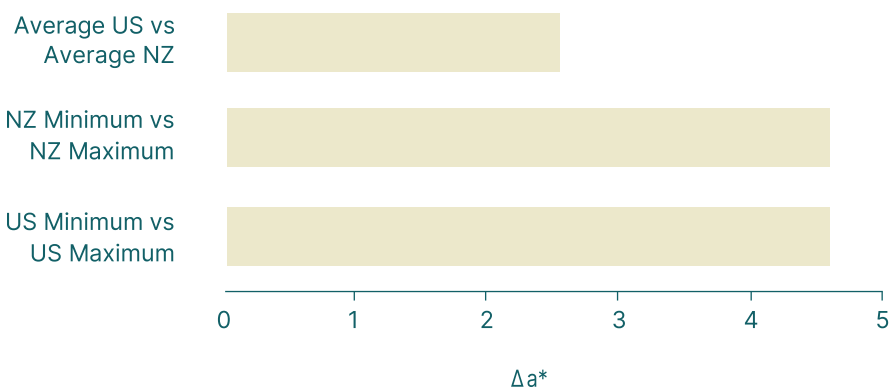


Figure 7: *Similarity of US and NZ powders measured by Δa*. Average US minus Average NZ Δa* value plotted against the Δa* values of the lowest a* minus the highest a* lots for both NZ and US origins.*

Figure 7 shows the Δa* between the average US powder and average NZ powder has a lower magnitude than the Δa* between the minimum a*-value lot and maximum a*-value lot from the same country of origin. Stated another way, the average US and average NZ powder are more similar in their green color than lots from the same country of origin are with each other.

CONCLUSION

The use of color science to understand color variability and compare different lots and grades of BSA provides more insight than simply describing the color by eye. New Zealand powdered BSA has traditionally been viewed as green by the eye compared to the US powders which are more yellow or amber. CIE L*a*b* values confirm that an average NZ powder is slightly greener than the US counterpart, but quantification revealed that half of the US and NZ lots are equally green based on a* values. The comparison of color variability based on Δa* values revealed the average US or NZ powder are more similar in green color to each other than lots from the same country of origin are with each other. Meaning that if the brown-to-green color variation of PHB US powders does not impact an application it can be safely assumed that brown-to-green PHB NZ powders should not impact that same application. This level of detailed understanding is only possible by using color science. When using color as a metric of product consistency color science provides a platform for objectively quantifying lot-to-lot variability. This allows the end user to determine what type of variability is acceptable for an application and if color variation correlates to performance. The variation or similarity can be converted from numbers to actual colors for assessing perceived color similarity or differences, so the analysis is appropriately contextualized. This approach proved critical to understanding how a plasma's country of origin could influence the final color of PHB BSA powders.

REFERENCES

EasyRGB. (2020). Color Math and Programming Code Examples. https://www.easyrgb.com/en/math.php
Johnstone, D. (2020). Lch and Lab colour and gradient picker. http://davidjohnstone.net/pages/lch-lab-colourgradient-picker

Lindon, J. C., Tranter, G. E., & Koppenaal, D. (2010). Encyclopedia of Spectroscopy and Spectrometry. London, UNITED KINGDOM: Elsevier Science & Technology.

X-Rite, P. L. (2016). A Guide to Understanding Color. https://www.xrite.com/learning-color-education/whitepapers/a-guide-to-understanding-color