Application note

Unbiased Color Analysis using UV-Visible Spectrophotometers

Introduction

Across a range of different applications, the color of a material can be an important quality assessment. For certain environments, like textiles and paint manufacturers, the color must match an established criteria to ensure product consistency. In other areas, like water analysis and pharmaceutical manufacturing,[1-4] the color of a product may indicate the presence of undesired substances. While the specific areas of study can vary greatly, the ability to rigorously assess a given sample's color is shared among these application spaces. Visual methods for determining color have been used in many of these industries; however, technician-to-technician biases inherently influence the analysis. This can lead to discrepancies between testing subjects and testing sites. As a result, it is often preferred to use an unbiased instrument-based method for precisely determining the color of a given product or sample.

The human eye is able to collect the light reflected off a surface, leading to the perception of color. Since the color range we can see covers the aptly named visible range of the electromagnetic spectrum (400 – 700 nm), UV-Visible spectrophotometers are often used for color analysis. These instruments are used to assess a material's response to light in the ultraviolet (UV) and visible spectral range. Based on the electronic structure of the material, light within this range is either absorbed, transmitted, or reflected off the material of interest. Given that color perception is based on the collected reflections from a material's surface, UV-Visible spectroscopy is an ideal technique for this analysis.

Once the visible spectrum of a given sample, either in liquid or solid form, is collected, there are a number of unique analysis methods that can be used to report the color. As there are many different industries which require this analysis, a multitude of different standardized methods exist which call for different color analysis procedures. Herein, a selection of these standard color analyses is detailed, and information pertaining to the industry standards required for these analyses are discussed. For these methods, special attention is focused on the use of UV-Visible techniques.

Soln	X	Y	Z	L*	a*	b*	C*	h*
Colorless	94.81	100.01	107.34	100.00	-0.01	-0.02	0.02	244.01
Red	75.84	58.73	58.16	81.14	45.43	4.42	45.65	5.55
Orange	74.90	69.67	31.65	86.83	18.97	44.17	48.07	66.76
Yellow	79.36	91.10	22.41	96.45	-13.49	75.23	76.43	100.17
Green	52.20	73.11	55.74	88.50	-40.63	19.40	45.02	154.47
Blue	50.94	62.30	92.59	83.07	-20.56	-19.59	28.40	223.61
Purple	57.31	54.70	72.89	78.87	13.84	-12.24	18.48	318.51

Table 1. Tristimulus, CIE L*a*b* and L*C*h* values for food dye samples.

Tristimulus and CIE Color Values

Some of the most common color analysis methods include the CIE *L*a*b** and CIE *L*C*h** color calculations which are based on the tristimulus color space (*X*, *Y* and *Z* color coordinates). These calculations take into account the light source the substance is expected to be viewed under (e.g. incandescent lightbulbs or daylight) as the color of an object can appear different under different illuminants. Unlike the tristimulus values alone, the CIE *L*a*b** and CIE *L*C*h** spaces are more uniform (see Figure 1), allowing for a better method of comparing the color of two different objects. Table 1 includes calculated tristimulus, CIE *L*a*b** and CIE *L*C*h** values for a set of food dye solutions (Figure 2a) based on the measured UV-Visible spectra (Figure 2b). More in-depth details about these calculations can be found elsewhere.[3-6]

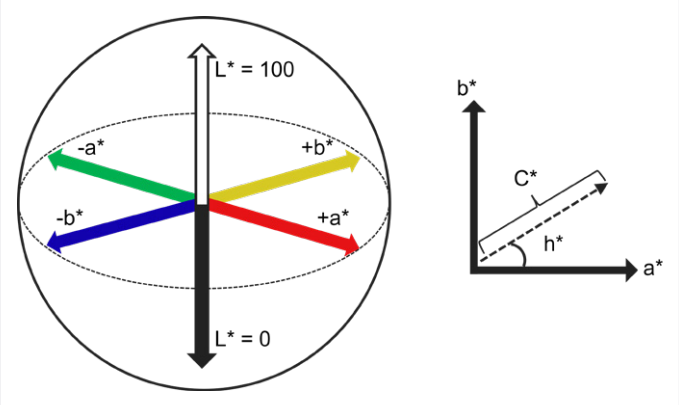

Figure 1. Visualization of CIE L*a*b* and CIE L*C*h* color space.

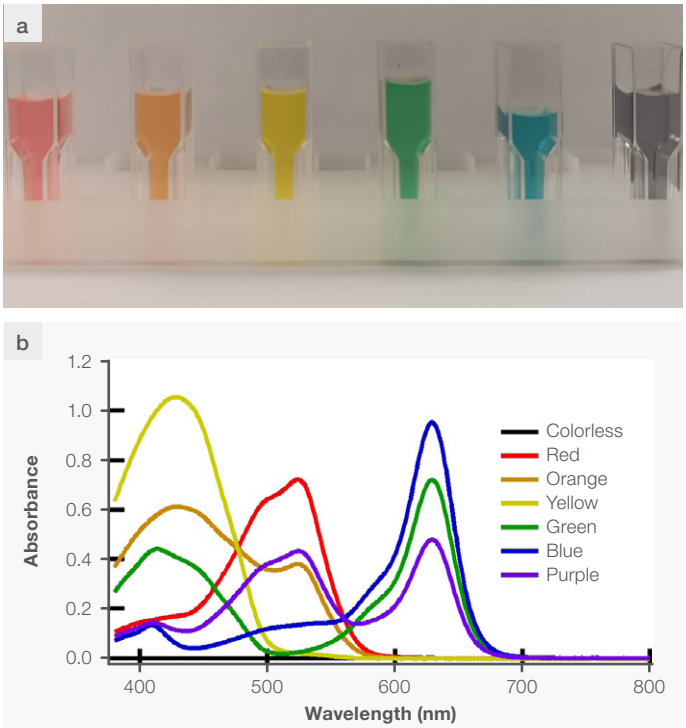

Figure 2. (a) Image of food dye solutions. From left to right: Red, Orange, Yellow, Green, Blue and Purple. (b) Spectra of food dye measured in a 1.0 cm path length polystyrene cuvette using a Thermo Scientific™ Evolution™ One Plus UV-Visible spectrophotometer.

In QA/QC environments, it can be helpful to ensure the color of a given product/material matches a specified standard. A useful method for comparison is through color difference calculations (ΔE^*),

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Equation 1.

where ΔL^*, Δa^* and Δb^* are the differences in the respective color values between a sample and reference material. In this analysis, the difference between the expected CIE L*a*b* value, often of a standard material, and that of the sample is used as a point of comparison. The resulting values are often used to determine if the color matches the chosen standard. They can also be used to determine if they meet a minimum or maximum color value criteria, as outlined elsewhere.[2,6]

In pharmaceutical environments, color analysis can be an important step in ensuring the quality of manufactured materials. For example, the presence of degraded materials can sometimes influence the color of the products, meaning simple color analysis can serve as a quick quality check. Consequently, both the European and United States Pharmacopeia (EP and USP, respectively) provide monographs outlining the requirements for analysis of color. These standard methods include reporting CIE *L*a*b** (EP and USP) or CIE *L*C*h** (EP only) color coordinates in addition to ΔE^* using a prepared matching solution.[2-4] For EP, the selected illuminant and observer angle must be C/2˚ while USP allows either C/2˚ or D65/10˚ to be used.[2,4]

The procedure for preparing matching solutions is further outlined in both USP and EP color analysis monographs.[2,4] The USP matching solutions are primarily yellow in appearance, while the EP monograph outlines solutions which cover other apparent colors as well, including brown (B), greenish-yellow (GY) and red (R).[4] USP <631> does outline the ability to follow EP procedures for preparing alternative matching solutions as well, if other color comparisons are needed.[2] Additionally, USP <1061> outlines practices for the analysis of solid substances. Under these circumstances, the diffuse reflectance of the material must be collected in place of the absorbance/transmission.[3] This measurement can be accomplished using a UV-Visible spectrophotometer equipped with an integrating sphere, as shown elsewhere.[6]

In addition to pharmaceuticals, the color of food products can also provide a valuable check for QA/QC environments. For example, the American Society of Brewing Chemists (ASBC) outlines a set of procedures for assessing the color of beer.[7] This method outlines two options, one in which two wavelengths (430 nm and 700 nm) are monitored, and one in which the CIE *L*a*b** color calculations are determined from a full spectrum UV-Visible measurement. While the first analysis method involves only two wavelengths—430 nm for assessing the color and 700 nm for confirming the lack of turbidity—the method does also indicate that the CIE *L*a*b** method provides a better procedure for determining the perceived color. Much like the pharmaceutical applications, this method requires the calculations be carried out using a 2˚ observer angle and illuminant C.[7]

Note, both the USP/EP monographs as well as the ASBC method indicate that samples should be filtered prior to analysis. For solution-phase measurements, this can be critical because debris, including insoluble or undissolved materials, can cause incident light to scatter. This scattering phenomenon can greatly influence the perceived transmission/absorbance of a solution as it is highly wavelength dependent. This can further lead to erroneous color values. By filtering out the particulates, including insoluble substances not easily observed by eye, this issue can be avoided. For carbonated beverages, like beer, degassing can also be vital as CO_2 bubbles can similarly scatter light.

Yellowness Index, Pt-Co and Gardner Color Values

While tristimulus and CIE *L*a*b** color values are common, other color analyses are called for in standardized protocols as well. One such method is the Pt-Co color scale, also referred to as Hazen or APHA color. This method is based on the coloration of solutions containing hexachloroplatinate and cobalt chloride.[1,8] Unlike the tristimulus analysis, this method is solely based on analyzing yellow solutions, as the Pt-Co standards used to create the scale are yellow in hue. This analysis began as a visual method of comparing color; however, by determining the yellowness index of the Pt-Co standard solutions, which is a value calculated from the tristimulus color coordinates,[8] a correlation between the measured spectrum and the Pt-Co color value can be determined. More details on yellowness index and Pt-Co color calculations are outlined elsewhere.[8-10]

Table 2 includes an example of the reported yellowness index values and Pt-Co color values for a set of yellow food dye solutions of varying concentration. Note that visually, as depicted in Figure 3a, the solutions are not strong or intense in coloration, yet the highest concentration is considered “Out of Range” for the Pt-Co color scale. This scale has an upper limit of 500. The spectrum of the “Out of Range” sample includes a maximum absorbance of ~0.25, which is well below the threshold for typical absorbance measurements. Based on these observations, it should be noted that the Pt-Co color range is typically used for samples with weak coloration.

Yellow Food Dye Solution	Yellowness Index	Pt-Co Color Value	Gardner Color Value
1	2.6	49.6	Out of Range
2	6.3	120.8	Out of Range
3	12.2	238.8	1.5
4	22.8	464.2	3.0
5	32.0	Out Of Range	3.9

Table 2. Yellowness index Pt-Co and Gardner color values for a set of yellow food dye samples of varying dye concentration.

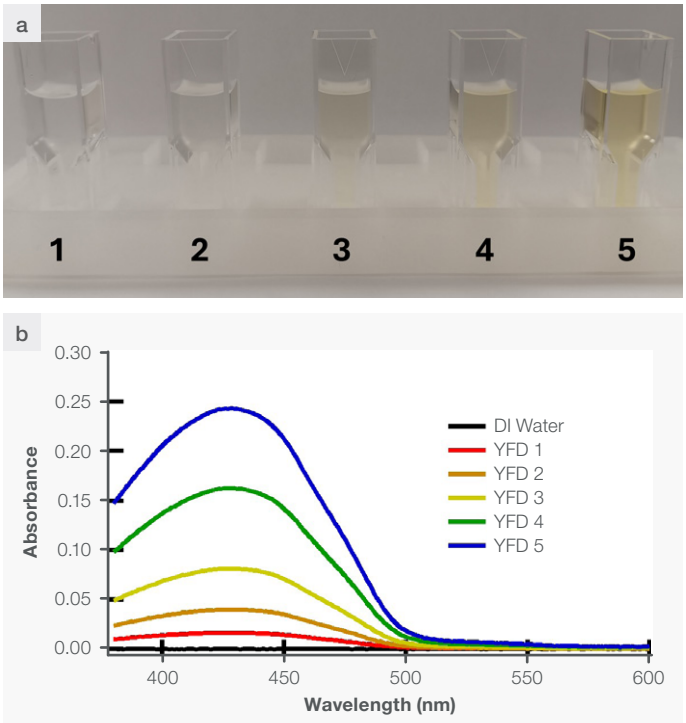

Figure 3. (a) Image of yellow food dye solutions. (b) Spectra of yellow food dye solutions measured in a 1.0 cm path length polystyrene cuvette using a Thermo Scientific Evolution One Plus UV-Visible spectrophotometer.

Having a specialized method for analyzing how yellow a substance appears is particularly useful for environmental applications. More specifically, this color scale is commonly found in water applications, including wastewater analysis.[1] In water sources, organic matter, like plants, can often be found. The degradation of this material can lead to the formation of compounds like humic acid, which imparts a yellow/brown coloration.[11] Consequently, the determination of the degree to which a water source appears yellow can suggest the presence of decayed material, leading to a better understanding of the quality of the water.

The American Public Health Association (APHA), in addition to the American Water Works Association and Water Environment Federation, recognize a set of standard methods for the analysis of water color, including the use of the Pt-Co color scale for comparison.[1] As this method was originally a visual comparison between samples and prepared Pt-Co standards, the procedure is still referred to as a visual method. However, the standard method does recognize that pre-programmed calibration curves for the Pt-Co standards can be used provided that Pt-Co standards are used periodically to confirm the calibration curve is appropriate.[1]

Note that the tristimulus color method described previously is also included as a standard color analysis method for water analysis as well. However, as this method can be used to assess colors other than and including yellow, it is useful for assessing water which may include other contaminants, like dyes, which impart an array of different colors. This reason behind the analysis substitution is outlined in the water analysis method on color.[1]

Though less frequently used, Gardner color analysis can also be used when analyzing yellow/brown materials. This analysis was originally developed to report the color of oils and resins and is still employed for these types of substances. For example, ASTM D6166 outlines procedures for assessing the color of pine chemicals, and it specifically calls for the use of Gardner color analyses alongside the procedure for determining these values.[12] This standard method specifically notes it is only applicable to liquid materials, meaning some samples, like resins, which may be solid at room temperature, will need to be heated to form a molten liquid.[12]

Table 2 includes the calculated Gardner color values for a set of yellow food dye solutions (Figure 3a). Note that for the lower concentration samples (yellow food dye solutions 1 and 2) a Gardner color value cannot be assigned, whereas Pt-Co color values cannot be assigned to the highest concentration sample (yellow food dye solution 5). As the range for this color scale has a maximum value of 18 color units, and the highest concentration sample measured here has a Gardner color value of 3.9, it is clear that, unlike the Pt-Co color scale, the Gardner scale is appropriate for samples with a stronger or more intense color.

Summary

The color of a finished product can have implications on the quality, both in terms of the appearance and the presence of degraded materials or contaminants. As shown herein, there exist many standardized methods for ascertaining the color of a given substance. While visual methods can be applied, the use of a UV-Visible spectrophotometer is widely accepted and is helpful in providing an unbiased technique for color analysis.

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