APPLICATION NOTE

# MEASURING THE BLUE, GREEN AND RED DYES IN CHOCOLATE CANDIES USING AN UV-VIS ABSORPTION AND REFLECTANCE SETUP

## INTRODUCTION

Color additives (or colorants) are dyes or pigments used to produce attractive colors, alone or through a chemical reaction, in products. Widely used in the food market, colorants belong to a group of compounds added to food products to maintain or restore color uniformity [1,2].

The food colorants market has been affected by several events, one of the most important being the number of food additives required after World War II. More recently, the food colorants market was affected by the preference of consumers for natural over artificial additives due to concerns of potential health hazards of artificial dyes in food [2,3].

Natural additives are defined as substances that exist in nature and are extracted using non-chemical processes.

**Keywords:**

UV-Vis Spectroscopy

Light Source LS-DWHP

FLEX Spectrometer

Diffuse Reflection Probe

Chocolate Candies

**Figure 1** – Spices, a source of natural colorants.

Traditional sources of natural food additives are plants, seeds, fruits, vegetables, algae, and insects whereas saffron, paprika, blueberry juice concentrate, red cabbage, carrot juice, and turmeric are some examples of natural colorants, see **Figure 1** [1-3].

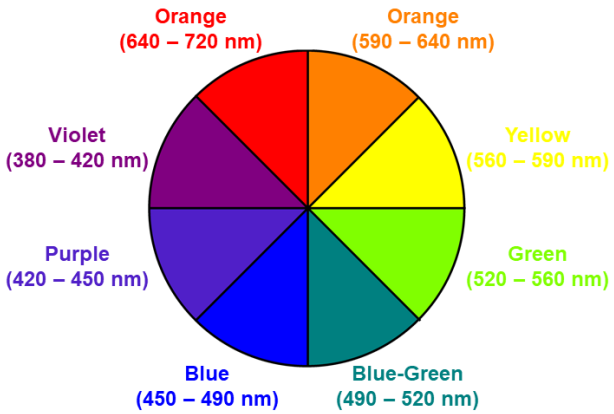



**Figure 2** – Color wheel.

From a spectroscopic perspective, food colorants share the property of absorbing ultraviolet and visible (UV-Vis) radiation.

The number of conjugated double bonds, which strongly affect the electronic delocalization, influences the wavelength of the absorbed radiation [4].

The color of natural additives ranges mostly from yellow to red with carotenes, riboflavin, anthocyanins, curcumin, betalain, and chlorophylls being some classic examples of these dyes [2].

White light is a mixture of all visible wavelengths that goes from around 380 to 720 nm. When white light shines on an object that appears colored, some wavelengths are absorbed while the remaining wavelengths are transmitted or reflected by the object.

Our color perception results from observing the wavelengths transmitted or reflected by this object. For an object to be colored under white light, it must absorb the light of its complementary color, see **Figure 2**.

In this application note, we combine a high-power deuterium tungsten-halogen (LS-DWHP) light source with FLEX spectrometer in an UV-Vis absorption and reflectance configuration (this requires the use of a reflection probe) to obtain the absorbance and reflectance spectra of the blue, green, and red dyes present in chocolate candies.

# MATERIALS & METHODS

## Reagents

- Blue, green and red dyes chocolate candies;
- Distilled Water;
- Barium Sulfate ($BaSO_4$, 99 %, Sigma-Aldrich);

## Instruments and Accessories:

### Absorbance measurements configuration

(see **Figure 3**)

- DWHP light source;
- Optical Fibers with 200 µm of core diameter;
- Standard cuvette holder set into an absorbance configuration;
- FLEX STD UV/Vis spectrometer (Slit: 10 µm)
- 10 mm absorption cuvettes in UV quartz;


**Figure 3** – DWHP Light source (1), FLEX spectrometer (2), and standard cuvette holder (3) were used measure the absorption of blue, green and red dyes in chocolate candies.

### Reflectance measurements configuration

(see **Figure 4**)

- DWHP light source;
- Reflection Probe (6 emitting and 1 detecting; 400 µm fibers; Stainless Steel Protection; 200 cm; UV-Vis Wavelength Range);
- FLEX STD UV/Vis spectrometer (Slit: 10 µm)
- Standard Probe Holder;
- Diffuse Reflectance Standards;

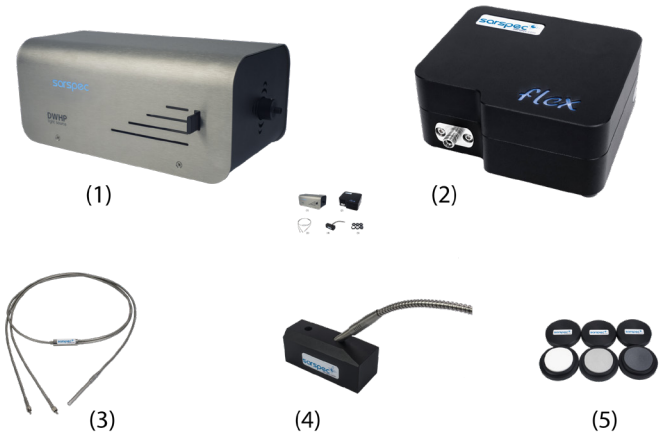



**Figure 4** – DWHP Light source (1), FLEX spectrometer (2), reflectance probe (3), standard probe holder (4), and diffuse reflectance standards (5).

# EXPERIMENTAL PROCEDURE

## Absorption measurements

**1.** Insert a blue chocolate candy in a 20 mL volumetric flask and add 7 mL of distilled water (Solution A). Be sure the chocolate candy is completely covered with water.

**2.** Once all the dye has been dissolved in the Solution A (the chocolate candy loses the blue color and becomes white), remove the candy. At this point, it might be necessary to wait around 10 to 15 minutes for large particles to precipitate.

**3**. Then, carefully take 1 mL from the top of Solution A and dilute it by adding 10 mL of distilled water (Solution B).

**4.** Take the 3 mL from solution B that must be completely homogeneous and measure the absorption spectra between 250 and 750 nm, using filtered distilled water as a reference. The solution in the flask should be colored but allow light to clearly pass through.

Table 1 – Instrument settings used for experimental absorption measurements.

| Parameter | Used Settings |
|---|---|
| Integration time (ms) | 2 |
| Average | 250 |
| Smoothing | 4 |

**5.** The LightScan software was used with the instrument's settings specified in **Table 1**.

## Diffuse Reflectance measurements

**1.** Remove the solid outer shell (around 50 mg) of a colored chocolate candy (blue, green, or red). Avoid carrying the inner chocolate part with the shell fragments.

**2.** Using an Agate mortar and pestle, grind the colored outer shell of the chocolate candy to a fine powder.

**3**. Fill the diffuse reflectance sample holder with the ground colored powder. Strongly press the colored powder with a glass cylinder until you get a small pellet.

**4.** Repeat steps 1, 2 and 3 for Barium Sulfate, which will be the reference for the diffuse reflectance measurements.

**5.** Put the reflection probe in the probe holder and use the 45-degree position. This optimizes the collection of the scattered radiation and minimizes the collection of specular radiation.

**6.** The LightScan software was used with the instrument's settings specified in **Table 2**.

Table 2 – Instrument settings used for experimental diffuse reflectance measurements.

| Parameter | Used Settings |
|---|---|
| Integration time (ms) | 100 |
| Average | 150 |
| Smoothing | 4 |

# RESULTS

The absorption spectra of the blue, green and red dyes solutions are presented in **Figure 5**.

The absorption spectrum of blue dye has a major peak with a maximum of around 630 nm and two minor peaks around 315 and 410 nm.

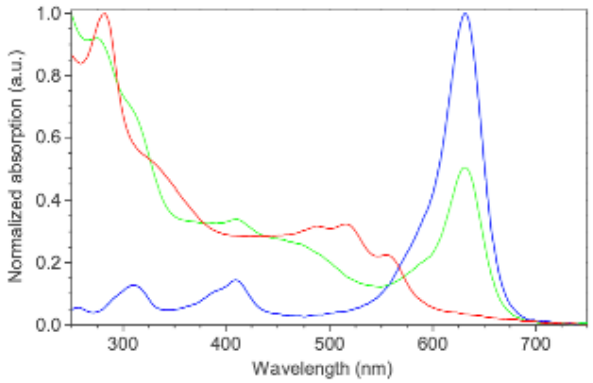



**Figure 5** – Normalized absorption spectra of the blue, green and red dyes, extracted from the chocolate candies.

The green dye, which results from a mixture between the blue and yellow dye, has the same two peaks at 410 and 630 nm corresponding to the wavelength region where the blue dye absorbs light, and a broad absorption band below 550 nm corresponding to the wavelength region where the yellow dye absorbs light. The red dye shows also a strong absorption molar coefficient between 250 and 600 nm.

The reflectance spectra of the blue, green, and red dyes pellets are presented in **Figure 6**. The reflectance spectra of the blue, green, and red dyes pellets have a wider but similar structure to the corresponding absorption spectra, see **Figure 5**. The peaks observed in the reflectance spectrum of all three dyes have a small shift to longer wavelengths, as a result of irregularities on the surface of the pellets.

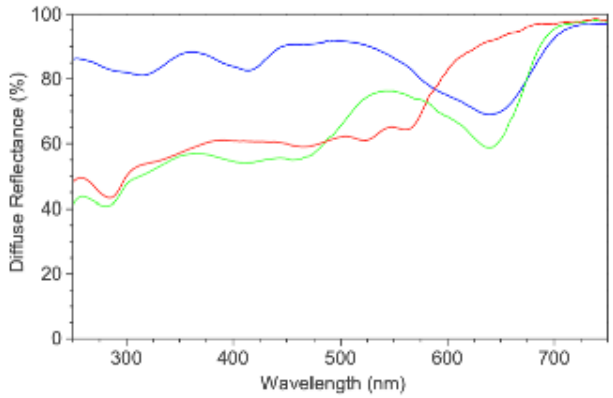


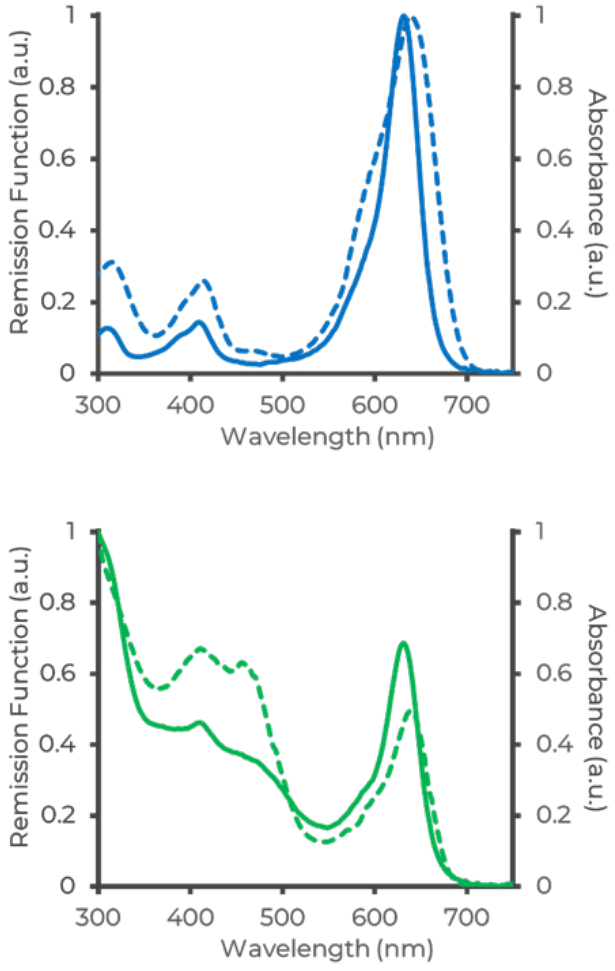

**Figure 6** – Reflectance spectra of the blue, green and red dyes pellets, extracted from chocolate candies.

Following what is discussed in the reflectance spectroscopic technique, when an inhomogeneous sample is illuminated, some radiation enters the sample and some are reflected from the surface. The amount of radiation that enters the sample undergoes scattering at a large number of points in its path, and some of it is absorbed.

The fraction of this radiation that enters and comes back out of the sample is the diffusely reflected component. This component is theoretically described by the widely-used Kubelka-Munk model [5,6], where the remission function $F(R_\infty)$ is defined as

$$F(R_\infty) = k/s = (1-R_\infty)^2/2R_\infty), \quad (1)$$

where, k corresponds to the absorption coefficient of the sample, s corresponds to the scattering coefficient and $R_\infty$ corresponds to the reflectance in an optically thick sample. Therefore, by calculating the remission function $F(R_\infty)$ using $R_\infty$ (here $\infty$ corresponds to a sample with a infinite thickness) from **Figure 6**, it is possible to approximately correlate these to the absorption spectral data, see **Figure 7**.



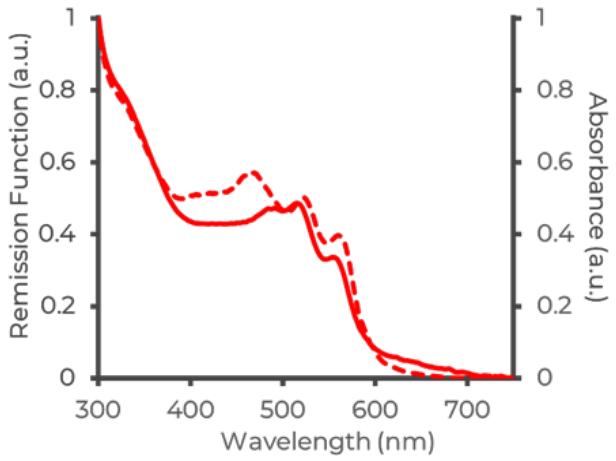



**Figure 7** – Remission function F(R_∞ ) as derived by equation 1 for (a) blue, (b) green and (c) red dyes obtained by ground-state diffuse reflectance technique (dash line), given in Figure 6. The absorption spectra of the corresponding dye solutions (solid line) were added for a peak structure and wavelength comparison.

Applying the Kubelka-Munk transformation to the diffuse reflectance spectral data (see **Figure 7**) yields spectra that are comparable to the absorption spectra of the solutions. However, differences in the wavelength peak positions and spectral structure are expected because of the differences in the physical state of the samples and because of the approximations in the Kubelka-Munk transformation.

## CONCLUSIONS

The absorption and diffuse reflectance spectra were obtained for blue, green, and red dyes of chocolate candies, with some peaks being comparable to both spectra. A reasonable superposition to the absorption spectral data can be observed when applying the Kubelka-Munk transformation to diffuse reflectance spectral data.

In this application note, we demonstrate that the combination of DWHP light source, FLEX spectrometer, and all the accessories required to assemble the absorption and diffuse reflectance configurations allows easy, fast, and accurate measurements of dyes in both liquid and solid states.

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