In recent years, non-invasive biomedical diagnostic applications have developed significantly. Non-invasive techniques often use optical methods such as fluorescence, scattering and absorbance to measure the optical properties of a biological specimen and to analyze physiological attributes. Absorbance spectroscopy in the visible range (400 – 700 nm) is used in the setup of this project. Measurement protocol is designed to obtain concentrations and optical densities of commercial food coloring. In addition, a new technique called spectral phasor analysis was used. We confirm that red and green food colors exhibit a linear absorption response up to about an absorbance of 1.5 and confirm that absorbance spectrum shape is independent of dye concentration in the linear regime. Finally, we quantify food-coloring mixtures in non-scattering media using spectral phasor analysis and determine mix ratios to within a few percent, about as good as the uncertainty in the volume measurement.
SPECTRAL PHASOR ANALYSIS ON ABSORBANCE SPECTRA FOR QUANTIFYING THE CONTENT OF DYE MIXTURES

A Thesis

Submitted to the

Faculty of Miami University

In partial fulfillment of

The requirements for the degree of

Master of Arts

by

Maha Mohammed Aljohani

Miami University

Oxford, Ohio

2016

Advisor: Dr. Paul Urayama

Reader: Dr. Karthik Vishwanath

Reader: Dr. Jennifer M. Blue

©2016 Maha Mohammed Aljohani
This Thesis titled

SPECTRAL PHASOR ANALYSIS ON ABSORBANCE SPECTRA FOR QUANTIFYING THE CONTENT OF DYE MIXTURES

by

Maha Mohammed Aljohani

Has been approved for publication by

The College of Arts and Science

And

Department of Physics

______________________________
Dr. Paul Urayama

______________________________
Dr. Karthik Vishwanath

______________________________
Dr. Jennifer M. Blue
# Table of Contents

ABSTRACT ................................................................................................................................. iii

TABLE OF CONTENTS .............................................................................................................. iv

LIST OF TABLES ....................................................................................................................... v

LIST OF FIGURES .................................................................................................................... v

ACKNOWLEDGMENTS .............................................................................................................. vi

CHAPTER I: Introduction and Motivation ................................................................................... 1

CHAPTER II: Theory and Background ....................................................................................... 2-5

CHAPTER III: Experimental Methods ....................................................................................... 6-11

CHAPTER IV: Results and Discussion ...................................................................................... 12-23

CHAPTER V: Conclusion .......................................................................................................... 24

REFERENCES ............................................................................................................................. 25
List of Tables

Table 3-1: The nine standards of the single color solutions (red and green) showing their relative concentration. The amount of dyes that pipetted from the previous sample is shown in the second column. The amount of de-ionized water is the last column of the table.

Table 3-2: The mixture of seven samples. Each sample has red and green color except the first and the last one which is pure color. M 1 is dilution of pure green dye with 2.7 ml of water. M 2 is starting mixture of the two red and green mixture dye. Each sample has the same value of deionizer water.

Table 3-3: Decreasing of the ratio of green dye is start from M2 to M6. M7 is pure red dye. Conversely, the red dye is start to increasing from M2 until it coms pure in M7. The deionized water is constant in all samples.

Table 4-1: the calculations of the green sample by using phasor and actual methods. The ratio \( \frac{B_2}{B_1} \) is 1.685.
List of Figures

Figure 2-1: Spectral phasor components of the calculations of Gaussian shaped spectra.

Figure 3-1: The setup that holds the cuvette between the fiber optics and the white light source in the same line and it shows how it’s close from each other.

Figure 3-2: Schema of the whole apparatus. The white light source emissions the light to alignment the cuvette. A fiber optics feeds the absorption to a spectrograph coupled to an ICCD.

Figure 3-3: ICCD contacted with spectrograph

Figure 3-4: Photo of the food coloring commercial.

Figure 3-5: It shows the mixture sample. The greenish is M1 and then starting to change the color by changing the amount of the mixture color for each sample. The last sample M7 is reddish.

Figure 4-1: Sample 6 [table 3-1] show that the intensity of the reference [Left top] that means before the light pass the sample and the intensity of the sample [Right top]. The absorbance [bottom] has absorbance at 500 nm.

Figure 4-2: Absorbance spectra of green dye solution. The visible light was absorbance in the red regime, which is around 540 nm. The sample number is prepared in table 1.

Figure 4-3: The spectrum of the Red dyes dilutions. The light is absorbed in the green regime at 450 and 650 nm.

Figure 4-4. Linearity of low dye concentrations that appears when plots are made F average of green and red food dyes vs. relative concentrations.

Figure 4-5: All samples of Green dye dilutions in least square difference.

Figure 4-6: The difference spectra of the two samples in linear and nonlinear regime. The top figure is the difference of the two samples 6 and 1. The bottom figure is the samples 6 and 9.
Figure 4-7: The least square of the red dye dilutions. The peaks that in the negative is the samples has high concentration.

Figure 4-8: The least square for sample with less concentration as show in (the bottom) and with high concentrations (the top).

Figure 4-9: Absorbance spectrum of the mixture dyes shows in the figure, the highest peak at 500 nm wavelength is the last sample.

Figure 4-10: Spectral Phasor at various dyes concentrations, No 1 is the high consecrations and No 9 is the lower concentrations. The error bars are standard deviations of repeated measurement.

Figure 4-11: Spectral phasor plot for dye mixtures. The error bars are standard deviations of repeated measurement.
Acknowledgements

I would first like to thank my thesis advisor Professor Paul Urayama. I have learned many things since I become Dr. Urayama’s student. He is spends very much instructing me hoe to search literature and how to collect data. I would like to thank my committee members, Dr. Jennifer M. Blue and Dr. Karthik Vishwanath, for their input, time and energy throughout this endeavor. To my lab-mates, Millicent Gikunda, Laxmi Risal, Madhu Gaire for their help and company in the lab.
Also, I would like to thank all faculty, staff and students at physics department at Miami University.
Finally, I must express my very profound gratitude to my parents Mohammed and Aisha and to my sisters Abeer, Reem and Aziza and brothers Abdullah, Talal and Sultan and my friends Ohud Alshammari and Somya Madkhaly for providing me with unfailing support and continuous encouragement throughout my study years. This accomplishment would not have been possible without them. Thank you.

Author
Maha Aljohani
CHAPTER I: Introduction and Motivation

❖ Introduction

Non-invasive medical techniques use external signal with no penetration of the skin and no damage done to tissue [1]. Common noninvasive approach uses optical methods such as absorbance, scattering and fluorescence measurements. Measurement of the absorption is important in turbid biological samples such as for clinical and pre-clinical applications related to diseases such as cancer [2].

Here, we develop applications of spectral phasor analysis to absorption spectroscopy. The absorbance properties of commercial food coloring were characterized in the visible light (400-700 nm) regime. More specifically, absorbance is the capacity of a substance to absorb light in a specified wavelength. It is equal to the logarithm of the reciprocal transmittance. Many studies use absorbance to measure the sample content particularly in biological samples [3]. Food coloring is a useful sample to measure the concentrations and to characterize absorption properties. It is also useful for testing whatever spectral phasor analysis can be used with absorption spectroscopy. Spectral phasor analysis is a recently developed approach for quantify the content of two components samples. Most of the researchers so far use this technique in fluorescence methods such as the quantitative measurement of NADH conformation in Dr. Urayama Lab [4], and other researches [3] [5] [6] [7] [8]. Here we show that it also works with absorption measurements.

❖ Motivation and Goals

This thesis has two goals,

Goal 1: Characterize absorbance properties of commercial food coloring. From the absorbance spectrum method, can be determine the property of any solutions such as food coloring.

Goal 2: Apply the spectral phasor analysis to quantify food-coloring mixtures in non-scattering media. By doing this we show that spectral phasor analysis is comparable of absorption spectroscopy.
CHAPTER II: Theory and Background

❖ Absorbance

Absorbance is one of the optical properties useful in many fields such as physics, chemistry and biomedical. Absorbance is useful for determining the concentration samples such as food coloring solutions. Usually it depends linearly on its concentration. The range of the visible wavelength is around 400-700 nm. Absorption results from the interaction between light and the molecules, raising the electrons from the ground state to an excited state. Light absorption is measure as function of wavelength or frequency, giving an absorbance spectrum. Dye identity is often characterized by its peaks and troughs (absorbance maxima and minima). The absorbance spectrum is a plot of absorbance and wavelength of the light that comes from the sample over specific wavelengths.

An objective of the analysis presented is to characterize absorbance properties of commercial food coloring. Absorbance is the measure the ability of a solution to absorb light. The definition of absorbance is,

\[ F = \log_{10} \frac{I}{I_0} \quad \text{...(2-1)} \]

where F is the absorbance. Note that most papers and books use the symbol A. F is used for this thesis because A will refer to the spectral phasor, which is described later in this chapter. \( I_0 \) is the incident light which is the reference, I is transmitted spectral radiant power through a sample.

The absorbance and scattering of the light are the foundations to measure the concentration of the sample and the dyes levels. During the passing of light through a mixture sample, there are two processes that occur which contribute to light attenuation, namely scattering and absorption. In a homogeneous sample, that is mixture with only a single-phase, the absorbance contributes greatly to attenuation.
Spectral Phasor Analysis

In the past, a spectral phasor analysis was used on in fluorescence spectroscopy. However, in this project, we show that it can also be applied to absorption spectroscopy. Spectral phasor analysis is a recently developed approach for quantifying the content of multi-component samples. It was developed by Fereidouni et al. [5, 11]. The method of the spectral phasors allows a spectrum to be represented as a point on the phasor plot.

A spectral phasor (A) is the first harmonic of the Fourier transform calculated over a wavelength interval,

\[ A = \sum_k F_k e^{\left(\frac{i2\pi k}{N}\right)} \ldots (2-2) \]

where \( N \) is the number of spectral channels, \( F \) is absorbance spectrum normalized to the integrated absorbance, and \( k \) is the channel number.

The real and imaginary components are

\[ \text{Re} \ (A) = \sum_k F_k \cos \left(\frac{2\pi k}{N}\right) \ldots (2-3) \]
\[ \text{Im} \ (A) = \sum_k F_k \sin \left(\frac{2\pi k}{N}\right) \ldots (2-4) \]

By assuming a measured spectrum \( F \) to be in a linear combination of two spectra \( F_1 \) and \( F_2 \), we define a fraction \( a \) that ranges from 0 to 1. Spectral phasors from a two-component system lay along line. Therefore, the spectral phasor is:

\[ A = \sum_k F_k e^{\left(\frac{i2\pi k}{N}\right)} \ldots (2-5) \]

\[ = \sum_k \left[a F_{1,k} + (1 - a)F_{2,k}\right] e^{\left(\frac{i2\pi k}{N}\right)} \ldots (2-6) \]

\[ = a A_1 + (1-a)A_2 \ldots (2-7) \]
A is a phasor that lies a fraction $a$ over a line joining between the two components $A_1$ and $A_2$.

The equation of the line on a plot of $\text{Im} (A)$ Vs $\text{Re} (A)$ is

$$\text{Im} (A) = \left( \frac{\text{Im}(A_1) - \text{Im}(A_2)}{\text{Re}(A_1) - \text{Re}(A_2)} \right) \text{Re} (A) + \left( \frac{\text{Re}(A_1)\text{Im}(A_2) - \text{Im}(A_1)\text{Re}(A_2)}{\text{Re}(A_1) - \text{Re}(A_2)} \right) \ldots \ldots (2-8)$$

The phasor positions of the pure samples ($a = 0, 1$) are the endpoints of the line. To find $a$, we calculate

$$a = \frac{\vec{u} \cdot (\vec{A} - \vec{A}_2)}{\vec{u} \cdot (\vec{A}_1 - \vec{A}_2)} \ldots \ldots (2-9)$$

where $\vec{u}$ is the direction vector of the fit line over different phasors. $\vec{A}$ is the spectral phasor that located between the $A_1$ and $A_2$ which as consider as a vector $(\text{Re} (A), \text{Im} (A))$. Thus, the first pure sample is $a = 1$ and the other pure sample is $a = 0$.

For explaining the relationship between the spectral phasors and shape of spectrum, Fig. (2-1) shows how phasor positions for Gaussian-shaped spectra form a crescent-shaped grid. The spectral phasor moves to the outer grid as the spectrum width narrows. In the limit of a delta function spectrum, a unit circle is formed as the maximum wavelength is shifted. Plot trajectories of the phasor for the maximum wavelength and width are roughly orthogonal along the negative real axis. In this region of the phasor plot, the maximum wavelength is centered in the wavelength interval. Here, $\text{Re} (A)$ becomes sensitive to the width of the spectrum and $\text{Im} (A)$ become sensitive to the peak wavelength of the spectrum.
Figure 2-1: Spectral phasor components of the calculations of Gaussian shaped spectra.

In two component systems, the variation of the absorption of the cross section and quantum yield is having to be consider to detection a relative concentration. To quantify that, the cross-section factor B needs to be known,

\[ a \alpha B_1 F_1 \ldots \ldots \quad (2-10) \]

\[ 1 - a \alpha B_2 F_2 \ldots \ldots \quad (2-11) \]

where \( F \) is a fractional concentration.

\[ \frac{a}{1-a} = \frac{B_1}{B_2} \frac{F_1}{F_2} = \frac{B_1 F_1 1}{B_2 (1-F_1)} \ldots \quad (2-12) \]

where \( F_1 + F_2 = 1 \), to find \( F_1 \) and \( F_2 \),

\[ F_1 = \frac{a B_2}{1-a+a \frac{B_2}{B_1}} \ldots \quad (2-13) \]

\[ F_2 = \frac{1-a}{1-a+a \frac{B_1}{B_2}} \ldots \quad (2-14) \]
where \( \frac{B_2}{B_1} \) is the ratio of the samples cross sections, which is found by determining the average absorbance.

CHAPTER III: Experimental Methods

❖ Instrumentation Set-Up

The experiment set up is shown in figure [3-1] [3-2]. The absorbance spectrum is measured by an intensified charge coupled device (ICCD) (model iStar 734, Andor) and spectra are obtained by using a spectrograph (model MS 125, Spectra-Physics/Newport) connected to the ICCD (figure [3-3]). The light emitted from the tungsten-halogen light source (QTH10, Thorlabs), passes through 1 cm a plastic cuvette. A 600 µm fiber optic is located in front of the cuvette to collect the signal. Spectra were averaged over five detector readouts in order to increase maximum single to noise ratio (SNR).

![Instrumentation Set-Up Image](image)

Figure 3-1: the setup that holds the cuvette between the fiber optics and the white light source in the same line.
Figure 3-2: schema of the whole apparatus. The white light source emissions the light to alignment the cuvette. A fiber optics feeds the absorption to a spectrograph coupled to an ICCD.

Figure 3-3: ICCD with spectrograph
Sample Solution Preparation

Deionized water is the reference for the absorption measurement. Stock dye solutions are prepared by adding 20 \( \mu l \) of commercial food coloring (fig [3-4]) to 2.98 ml deionized water. The stock concentration is called C, which is the first standard as shown table [3-1]. The green and red food coloring was diluted nine times fig [3-4]. The total amount that filled out of each sample was measured in a cuvette at room temperature. As shown in Table [3-1], the protocol was a series dilutions for the single color solution, each time pipetted from the previous cuvette. In mixture dyes sample (Table [3-3]), each one pipetted from the stock solution. The first objective is to characterize the samples of red and green dyes.

The calculation of the relative concentration of each sample was measured as the following example. We choose sample 2 to show how relative concentration was calculated:

\[
0.666 \times \left( \frac{1.9 \text{ ml}}{1.9 \text{ ml} + 1.1 \text{ ml}} \right) = 0.422 \text{ C} \quad \ldots \quad (3-1)
\]

Here .666 is the relative concentration of the previous sample and 1.9 ml is the amount of the dye sample that pipetted from the previous one to add it to the cuvette that was filled out with 1.1 ml of deionized water.
<table>
<thead>
<tr>
<th>Standard</th>
<th>Relative Concentration</th>
<th>Series of Samples dyes</th>
<th>Deionized water</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Stock) 1</td>
<td>1</td>
<td>0.02 ml</td>
<td>2.98 ml</td>
</tr>
<tr>
<td>2</td>
<td>.666</td>
<td>2 ml from the stock</td>
<td>1 ml</td>
</tr>
<tr>
<td>3</td>
<td>.422</td>
<td>1.9 ml</td>
<td>1.1 ml</td>
</tr>
<tr>
<td>4</td>
<td>.253</td>
<td>1.8 ml</td>
<td>1.2 ml</td>
</tr>
<tr>
<td>5</td>
<td>.143</td>
<td>1.7 ml</td>
<td>1.3 ml</td>
</tr>
<tr>
<td>6</td>
<td>.076</td>
<td>1.6 ml</td>
<td>1.4 ml</td>
</tr>
<tr>
<td>7</td>
<td>.038</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>8</td>
<td>.017</td>
<td>1.4 ml</td>
<td>1.6 ml</td>
</tr>
<tr>
<td>9</td>
<td>.007</td>
<td>1.3 ml</td>
<td>1.7 ml</td>
</tr>
</tbody>
</table>

Table 3-1: The nine standards of the single color solutions (red and green) showing their relative concentration. The amount of dyes that pipetted from the previous sample is shown in the second column. The amount of de-ionized water is the last column of the table.
Table 3-2 shows the mixture samples, and the fraction of green.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Fraction Green</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green Solution M (1)</td>
<td>1</td>
</tr>
<tr>
<td>M(2)</td>
<td>.833</td>
</tr>
<tr>
<td>M(3)</td>
<td>.666</td>
</tr>
<tr>
<td>M(4)</td>
<td>.500</td>
</tr>
<tr>
<td>M(5)</td>
<td>.333</td>
</tr>
<tr>
<td>M(6)</td>
<td>.166</td>
</tr>
<tr>
<td>Red Solution M (7)</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3-2: The mixture of seven samples. Each sample has red and green color except the first and the last one which is pure color. M 1 is dilution of pure green dye with 2.7 ml of water and .02 ml undiluted food coloring. M 7 is dilution of pure red dye with 2.7 ml of water and .02 ml undiluted food coloring. M 2 is starting mixture of the two red and green mixture dye. Each sample has the same value of deionizer water.
Figure 3-5: it shows the mixture sample. The greenish is M1 and then starting to change the color by changing the amount of the mixture color for each sample. The last sample M7 is reddish.

The pure-color samples M1 and M7 were prepared to have roughly equal absorbances of less than 1.

The mixture of the two dyes was starting from M 2, is mixing .25 ml of green dye with .05 ml of red dye. The amount of dyes that adding to each M samples was pipetted from the stock solutions of the greed and red dyes. Table [3-3] shows the ratio of each mixing sample.

<table>
<thead>
<tr>
<th>Solutions</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
<th>M7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>.3 ml</td>
<td>.25 ml</td>
<td>.2 ml</td>
<td>.15 ml</td>
<td>.1 ml</td>
<td>.05 ml</td>
<td>0</td>
</tr>
<tr>
<td>Red</td>
<td>0</td>
<td>.05ml</td>
<td>.1ml</td>
<td>.15ml</td>
<td>.2ml</td>
<td>.25ml</td>
<td>.3ml</td>
</tr>
<tr>
<td>Deionized water</td>
<td>2.7ml</td>
<td>2.7ml</td>
<td>2.7ml</td>
<td>2.7ml</td>
<td>2.7ml</td>
<td>2.7ml</td>
<td>2.7ml</td>
</tr>
</tbody>
</table>

Table 3-3: Decreasing of the ratio of green dye is start from M2 to M6. M7 is pure red dye. Conversely, the red dye is start to increasing from M2 until it comes pure in M7. The deionized water is constant in all samples.
CHAPTER IV: Results and Discussion

❖ Result and Discussion.

In this chapter, the results of the experiment are presented. However, the result of each goal will be shown in separate sections. Figure [4-1] shows a representative reference [left top] and dye transmission spectrum [right top] and the absorbance of Sample 6 from the red dye dilutions.

![Graphs showing transmission and absorbance spectra](image)

Figure 4-1: Sample 6 [table 3-1] show that the transmission spectrum of the reference [left top] and the transmission spectrum of the sample [right top]. The absorbance spectrum [bottom] has a peak absorbance at 500 nm.
Note that the intensity is reverse of the absorbance. There is no peak around 500 nm in figure 4-1 [right top]. While the peak show clearly in Figure 4-1 [bottom]. The spectra is smoothed in the reference spectra.

Absorbance Spectrum:

Fig 4-2 shows the absorbance spectra of green dye dilutions. The absorbance was the smallest around 540 nm with peaks on two sides at around 650 nm and 450 nm. Note that there is a change in spectrum shape as concentrations are increased, indicating a possible nonlinear effect.

![Figure 4-2: Absorbance spectra of green dye solution. The visible light was absorbance in the red regime, which is around 540 nm. The sample number is prepared in table 3-1.](image)

The samples with high concentrations such as sample 1,2 and 3 is more noise so the data point is discrete set due to liner interpolate was using from the Origin program.

The red food coloring was also measured. Figure 4-3 shows the spectrum of nine samples from 400 to 650 nm. Also, the peak is shifting in the sample at high concentrations, indicating a nonlinear effect. The red coloring absorbs maximally in the green regime around wavelength 500 nm.
Figure 4-3: the spectrum of the Red dyes dilutions. The light is absorbed in the green regime at 450 and 650 nm.
Analysis of the errors:

Errors analysis is important part to consider. There are different sources of the error such as the pipetting error, the different amount of a dye concentrations in each sample, the difference of the light source and range of the optical system in the set up for each dye, and so on. However, the big issue is the error that comes from the noise of the light source. There are a few percent uncertainty to absorbance measurement which is normally less than 5%. Therefore, the standard deviation is the uncertainty of the absorbance of several spectral data taken frequently on the same sample.

In figure [4-4], the absorbance of each sample is averaged over all wavelengths. The red and green dyes exhibit a linear absorbance response up to F= 1.5, above when nonlinearity is observed. Nonlinearity starts at around sample 4 for each case.

Figure 4-4. Linearity of low dye concentrations that appears when plots are made F average of green and red food dyes vs. relative concentrations.
Minimization of least square difference:

In the linear regime, the absorbance spectrum shape should be independent of dye concentration. To confirm this, difference spectra were calculated using the minimization of least-square difference method on green and red food coloring dilutions shown in figures [4-5][4-6]. The error function is defined as

\[ Q(a) = \sum_{\lambda} (F_1(\lambda) - a F_2(\lambda))^2 \]  …… (4-1)

where \( Q \) is error function, \( F_1 \) is the reference, \( F_2 \) is the spectrum to scale, and \( a \) is found from

\[ \frac{dQ}{da} = 0 \]  …… (4-2)

Solving for \( a \) gives.

\[ a = \frac{\sum F_1 F_2}{\sum F_2^2} \]  …… (4-3)

In Figure 4-5, difference spectra of all green dye samples are shown. Sample 6 is our reference to find the least square. Figure [4-6] shows the shape of two difference spectra; the top figure is sample 1 that has high concentrations, located in the nonlinear regime. The figure in the bottom has low concentration and is in the linear regime.
Figure 4-5: All samples of Green dye dilutions in least square difference.

Figure 4-6: the difference spectra of the two samples in linear and nonlinear regime. The top figure is the difference of the two samples 6 and 1. The bottom figure is the samples 6 and 9.
Also the same process was used with the red dye. It shows that the difference spectra in the linear regime is small. The difference increase at high concentrations with more noise observed.

Figure 4-7: the least square of the red dye dilutions. The peaks that in the negative is the samples has high concentration.
Figure 4.8: The least square for sample with less concentration as shown in (the bottom) and with high concentrations (the top).
Food-Coloring Mixtures Spectrum:

The next step is working with food-coloring mixtures. Red and green colors were used to make the mixture. The concentrations of the sample was prepare with different ratios as show in table [2-3]. The first standard is the pure green and the last one is the pure red. Isosbestic points are observed around 440 and 570 nm due to the superposition for two absorbance spectra.

Figure 4-9: Absorbance spectrum of the mixture dyes shows in the figure, the highest peak at 500 nm wavelength is the last sample
Spectral Phasor Analysis:

Spectral phasor analysis for single dyes:

The absorbance for single dye and mixtures were quantified by using spectral phasor analysis.

![Graph of Spectral Phasor analysis for single dyes.]

Figure 4-10: Spectral Phasor at various dyes concentrations, sample 1 is the high concentration and sample 9 is the low concentration. The error bars are standard deviations of repeated measurement.

Figure [4-10] (left) represents the green dye. It shows the two phasor component (real and imaginary). The lower concentrations are on the right side and higher concentrations in on left side. Error bars are the standard deviations of the four times spectral measurement.

Figure [4-10] (right) shows a spectral phasor plot of the red dyes. The phasor start to shift from the top left side, which is less concentrations to the bottom right side, which is the high concentrations. In each case, the spectra phasors cluster at low concentrations (linear response region) consistent with the lack of concentration dependence in spectrum shape.
Spectral phasor analysis for mixture dyes:

Note we show that mix ratios can be determined using spectral phasor analysis. Figure [4-11] shows a plot of the spectral phasors for the mixtures. It shows that the pure green start from the right bottom side the first point in the left side is the pure red and between that started mixture dye. By comparing figure [4-11] and figure [4-10], the pure green (sample 1) is located in the same regime with low concentrations samples in figure [4-10] (left). Also, the pure red (sample 7) is located the same regime with the least concentrated sample in figure [4-10] (right).

Figure 4-11: Spectral phasor plot for dye mixtures. The error bars are standard deviations of repeated measurement and may be smaller than the symbol’s size.
Figure [4-11] shows that how the spectra phasor analysis is applied to the absorbance of the dye mixtures. To see the distance between each sample, spectral phasors are calculated as shown in CHAPTER III. Table [4-1] shows the green-dye fraction calculated using spectral phasor analysis and using pipetted values. On average, the two methods agree to within 3.2%, which is comparable to the uncertainty in the volumes measured by the pipettes.

<table>
<thead>
<tr>
<th>Samples</th>
<th>$a$</th>
<th>Actual(Green)</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>.778</td>
<td>.833</td>
<td>0.08</td>
</tr>
<tr>
<td>3</td>
<td>.526</td>
<td>.666</td>
<td>-.218</td>
</tr>
<tr>
<td>4</td>
<td>.381</td>
<td>.500</td>
<td>2.00</td>
</tr>
<tr>
<td>5</td>
<td>.238</td>
<td>.333</td>
<td>3.74</td>
</tr>
<tr>
<td>6</td>
<td>.115</td>
<td>.166</td>
<td>7.94</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4-1: the calculations of the green sample by using phasor and actual methods. The ratio $\left(\frac{B_2}{B_1}\right)$ is 1.685.
CHAPTER V: Conclusions

The first goal was to characterize absorbance properties of commercial food coloring. We confirmed that red and green food colors exhibit a linear absorbance response up to about an absorbance 1.5. Also, we confirmed that absorbance spectrum shape is independent of dye concentration in the linear regime by calculating difference spectra and by mapping spectra onto a phasor plot.

In the second goal, we test whether spectral phasor analysis can be used to quantify food-coloring mixtures. Spectral phasors of red/green mixtures appear collinear, consistent with a two-component assumption. We show that mix ratios can be quantitatively extracted using spectral phasor analysis which agrees with known pipetted values of the mix ratio.
References:


