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A SIMPLE SPECTROSCOPE FOR STUDYING OF CHLOROPHYLL AND HAEMOGLOBIN – COMPLEMENTARY SUBSTANCES

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ABSTRACT:
A simple spectroscope is constructed using a transparent CD Rom as a diffraction grating for dispersion of light. Red laser (wavelength 650 nm), green laser (wavelength 532 nm) and blue laser (wavelength 445 nm) are used as reference wavelengths on a spectrum scale. A super bright white light LED was used as a light source for the measurement of a spectrum. A digital camera was used to take a picture of an absorption spectrum. Chlorophyll solution was extracted from green leaves using methyl alcohol. Haemoglobin dilute solution from human blood was also prepared for the measurement of an absorption spectrum. Reference absorption spectra of some samples are compared with the results from this simple spectrometer, showing similar results. Chlorophyll and Hemoglobin absorb different bands of light in the spectrum, and it seems that they are complement each other to complete white light spectrum. However, both Chlorophyll and Hemoglobin have high absorption around 400-450 nm which is in the range of blue color. Chlorophyll absorbs blue laser light and emits red light by fluorescence. Using a UV light source shining into chlorophyll solution, we can see red color from chlorophyll solution and a red leaf. This is because of fluorescence of chlorophyll when absorbing UV light.

Keywords:- Spectroscope, Absorption Spectrum, Chlorophyll, Haemoglobin, fluorescence.

INTRODUCTION :

Spectroscope is commonly used to study the wavelength emitted by a light source. The light passes through a narrow slit then a grating and is dispersed into a spectrum as shown on Fig. 1. The spectra to the right of the slit show the colors in the order of their wavelengths the extreme red and violet have wavelengths of about 760 nanometers and 390 nanometers respectively.

Normally in the standard spectrometer, we use white light passing through a slit and diffraction grating to get the spectrum of visible light for analysis of an absorption of substances. Standard commercial spectrometers are expensive. We can use alternative way to construct a simple and low-cost spectrometer by using a transparent CD rom as a diffraction grating for using in the classroom. A red laser with wavelength of 670 nm, a green laser with wavelength of 532 nm, and a blue laser with wavelength of 445 nm can be used as standard reference wavelengths of the spectrum.

It is interesting to study about Chlorophyll and haemoglobin - the complementary substances. They are two essential pigments for plant and animal life, respectively. Chlorophylls are found in photosynthetic organisms such as plants, algae, and cyanobacteria, whereas haemoglobin is found in vertebrate red blood cells. The structures of haemoglobin and chlorophyll are similar. The main distinction is that haemoglobin is based on iron (Fe), whereas chlorophyll is based on magnesium (Mg) as shown in Fig.2. Both are considered fundamental molecules of life because

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photosynthesis is the most fundamental function of plants, and plants cannot perform photosynthesis without chlorophyll. On the other hand, haemoglobin in blood is responsible for transporting oxygen throughout the body. Absorption spectra of Chlorophyll and Haemoglobin are shown in Fig.3. Both are responsible for color, as chlorophyll gives plants green, and haemoglobin gives blood red. They absorb different bands of light in the spectrum, and it seems that they are complement each other to complete white light spectrum.

This work is to construct a simple spectroscope using a CD Rom disk as a grating for studying of Chlorophyll and Hemoglobin absorption spectra.

MATERIALS AND METHODS

Making Diffraction Grating from a CD

A normal writable CD can be used as a diffraction grating for a spectroscope.

Firstly, scratch on the painted side on the CD then cover the pained side of the CD with a sticky tape. Gently remove the sticky tape. A CD will become transparent and can be used as a spectroscope grating as shown in Fig. 4.

Measurement of Absorption Spectrum of Chlorophyll and haemoglobin

In this experiment we used 3 different color lasers, red, green, and blue laser for accurate reference wavelengths of the spectrum scale. Fig. 5 shows an experimental setup for absorption spectrum measurement. We used super bright white LED as a light source for the measurement. A digital camera was used to take a picture of an absorption spectrum. Chlorophyll solution was extracted from green leaves using methyl alcohol. Haemoglobin dilute solution from human blood was also prepared for the measurement.

RESULTS AND DISCUSSION :

A red laser with wavelength of 670 nm, a green laser with wavelength of 532 nm, and a blue laser with wavelength of 445 nm were used as standard reference wavelengths of the spectrum, as shown in Fig. 6.

From experiment result in Fig. 7, we can see that Chlorophyll has high absorption range of around 400 - 450 nm and 650 – 680 nm. Low light absorption of Chlorophyll has a range of around 450 – 650 nm which is mainly around the range of green color. Haemoglobin has high an absorption range of around 400 - 650 nm and low absorption around 650-700 which is in the range of red color. Chlorophyll and Hemoglobin absorb different bands of light in the spectrum, and it seems that they are complement each other to complete white light spectrum. However, both Chlorophyll and Hemoglobin have high absorption around 400-450 nm which is in the range of blue color.

In stead of using a bright white light from LED, we used a strong intensity blue laser with a wavelength of 445 nm passing through a chlorophyll sample cuvette. Chlorophyll does not absorb blue laser light much. We can see blue laser light coming out from chlorophyll solution. There is only blue laser light of wavelength of 445 nm input, but we can see red light coming out from a sample as shown in Fig. 8. We found that chlorophyll absorbs blue laser light and emits red light by fluorescence. To confirm this result, we tried another experiment using ultraviolet light shining in chlorophyll solution as shown in Fig.9. When we use white light shining on chlorophyll solution or a green leaf, we can see green color. But if we use UV light, we can see red color of chlorophyll solution and a red leaf. This is because of fluorescence of chlorophyll when absorbing UV light.

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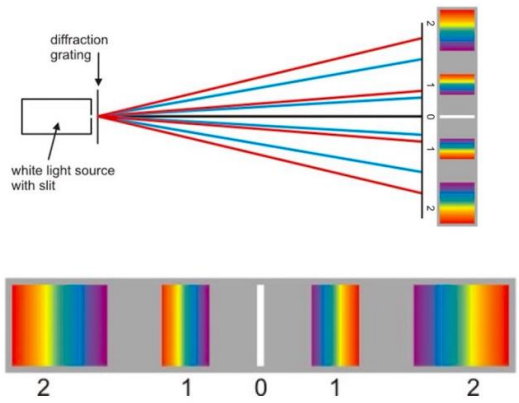

Fig. 1 Principle of Diffraction Grating Spectroscope

Fig. 2 Chemical structures of Chlorophyll and Haemoglobin.

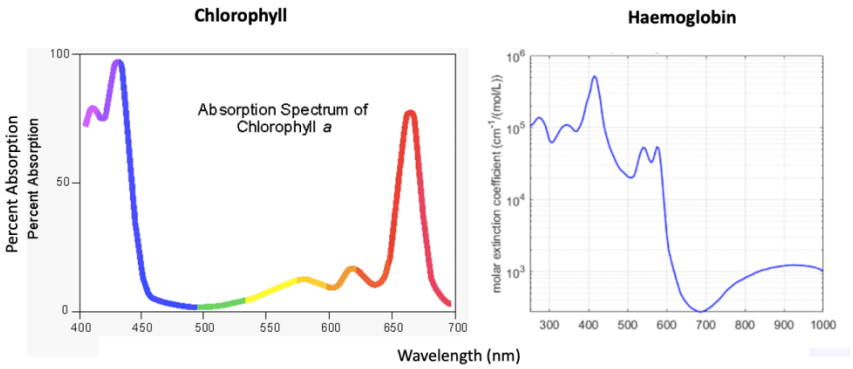

Fig. 3 Absorption spectra of Chlorophyll and Haemoglobin.

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Fig. 4 showing how to make a CD as a spectroscope grating.

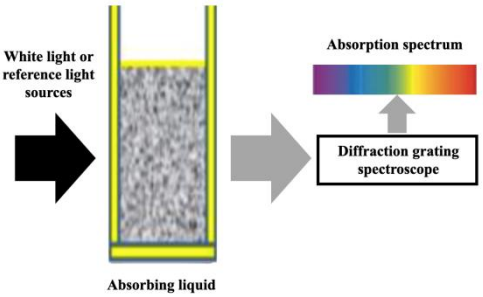

Fig. 5 Showing an experimental setup for absorption spectrum measurement.

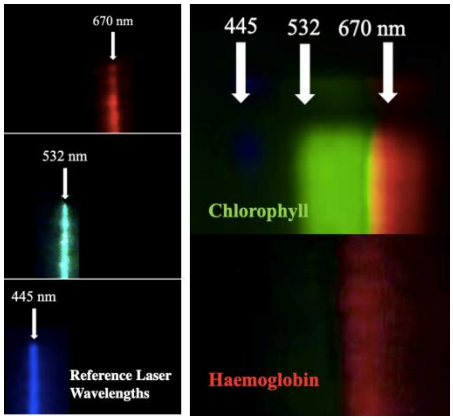

Fig. 6. (Left: showing spectrum of reference laser. (Right) showing absorption spectra of Chlorophyll and Haemoglobin.

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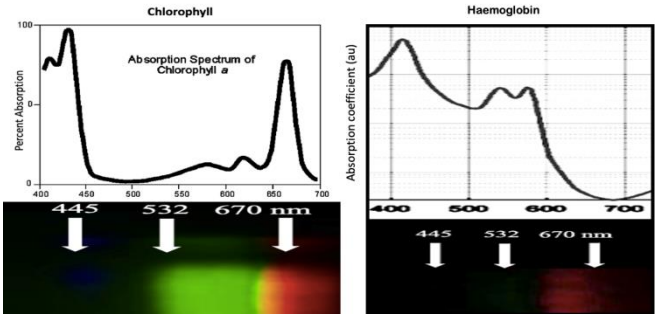

Fig. 7. Showing absorption spectra of Chlorophyll and Haemoglobin compared to the images from a spectroscope.

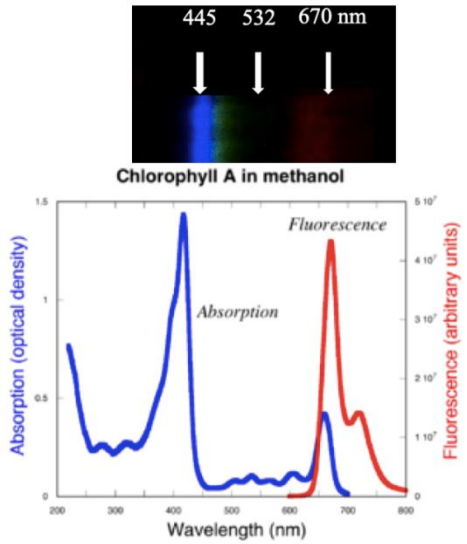

Fig. 8. Showing absorption spectrum and fluorescence spectrum of Chlorophyll. comparing to the image from a spectroscope.

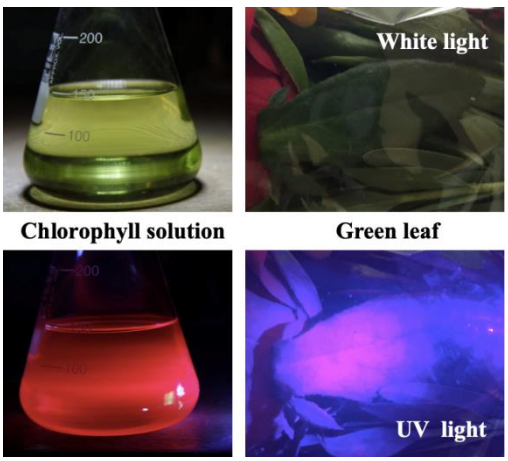

Fig. 9. Showing fluorescence of chlorophyll when absorbing a UV light.

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