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Measuring Dyes Concentration Using a Low-Cost Visible-Light Spectrophotometer

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Abstract

A low-cost, RGB LED-based visible-light spectrophotometer was designed to measure dyes concentration. Dyes are widely used as indicators or coloring agents in different applications and knowing their concentration is an essential part for many studies. The proposed spectrophotometer provides many functionalities that clones the traditional expensive spectrophotometers for a budged price under $50. It was aimed to provide a versatile tool for instructors and educators to teach their students the fundamental concepts behind spectrophotometry. Malachite green, methyl red, and methyl orange dyes were chosen to be good samples to show the integrity of the proposed spectrophotometer in terms of accuracy, repeatability, and sensitivity as compared to a conventional measurement.

Keywords: 3D Printing, Arduino, color sensor, dyes, RGB LED, visible-light spectrophotometer.

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1- Introduction

A spectrophotometer is a device that measures the intensity of light passing through a specified medium (usually a liquid) at a given wavelength. It was found that the intensity of the incident light depends on the distance being traveled by the light and the concentration of the absorbing medium. This fact was correlated clearly by Beer-Lambert law [1]. Visible-light spectrophotometer uses the visible spectrum of electromagnetic radiation which has a wavelength approximately ranging from 380 up to 750 nm. Spectrophotometers can also be made to cover the ultraviolet (190-380 nm) or near-infrared (750-2500 nm) non-visible regions of the electromagnetic spectrum. In fact, many commercial spectrophotometers were designed to cover all the mentioned zones, tagged as UV-Vis-NIR spectrophotometers.[2–5]

A typical spectrophotometer would be composed basically from four parts: (1) light source – covering the required wavelength of measurements; (2) Monochromator – to decompose the light source into its basic components via a prism or a grating material after being collected by the collimator (lens assembly to concentrate the light); (3) slit – allowing the passage of the selected decomposed beam; (4) photocell - to sense the intensity of the light beam after being passed through the absorbing medium. A rectangular container usually made of plastic or glass called a *cuvette* were used to hold the sample to be measured. The cuvette has two parallel transparent sides allowing the passage of the light through.

The length between the sides affects the value of the measured absorbance (e.g. 10 mm in case of standard cuvettes). The cuvette should be placed into a dedicated holder in the measuring chamber in such a way that the transparent sides always face the light source. Cuvettes must always be clean, free from fingerprints or any dirt to prevent any interference that can be a source of error (refer to Fig. 1). All the parts were adjusted and calibrated carefully by the manufacturers to ensure measurement accuracy. Off course, additional mechanical and electronic components were also included in their designs to achieve the target requirements [6–8].

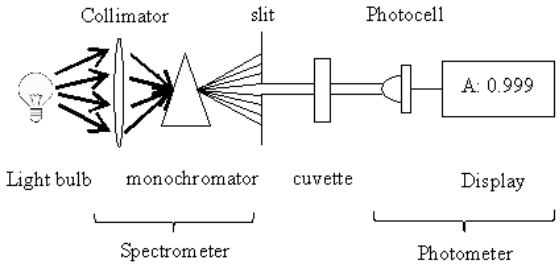

Fig. 1. Components of a typical spectrophotometer

To make a measurement, the intensity of light passing through solute free medium (called the *blank*) should be measured first. The ratio of the intensity in the presence (I) and absence of the chemical solute (I_0) is called the *transmittance* (T). The amount of light being absorbed by the solute is called the absorbance (A) and it is related to the transmittance by the relation:

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$$A = \log(1/T) \quad (1)$$

Multiplying both the nominator and denominator inside the logarithmic expression in equation 1 by 100, gives:

$$A = 2 - \log(\%T) \quad (2)$$

Spectrophotometers usually gives the operator the option to display the measurements results either by %T or by A. According to Beer-Lambert Law, at low concentrations, the amount of light being absorbed by the absorbing medium is proportional linearly with its concentration according to some thresholds:[9]

$$A = \varepsilon b c \quad (3)$$

in which ε is the molar attenuation coefficient (l/mol.cm) that reflects how far the medium species can attenuate the light, also known as *absorptivity*, *b* is the path length (cm), and *c* is the concentration of the absorbing species (mol/l).

Absorbance measurements can either be used to *qualify* or *quantify* chemical species. To qualify a given sample, absorption spectrum curve must be established. Absorption spectrum is a plot that shows the relationship between the absorbance and the wavelengths scanned over the tested sample. The curve represents a characteristic feature for the absorbing species and the wavelength in which maximum absorbance (λ_{max}) occur is a key feature to identify the species.

To quantify the concentration of the unknown samples, calibration curve should be constructed between sample already known concentrations and their absorbance. Measurements should be carried out at a maximum absorbance wavelength (λ_{max}). A linear relationship should be established according to equation 3. Unknown concentration can be obtained then by projecting a line from absorbance over the calibration curve and reads the corresponding concentration value from the other axis.

The slope of the line represents the molar absorptivity times the path length, *b*. The path length value is determined from the cuvette light path which can range from 1 mm up to 100 mm depending on the application area of study. Standard cuvettes have a light path length equal to 10 mm.

Spectrophotometers are extensively used devices and have broad applications in analytical chemistry, biochemistry, clinical chemistry, physics, food engineering, and water analysis.[10–13] However, these instruments are expensive, requires well trained and qualified operators, and they are generally large due to the size and arrangement of the optical filters and mechanical parts. So, they are not always authorized for students to carry out their measurements and calculations directly even in developed countries. If so, then they might do that after training or under supervision of authorized key person.

Manufacturers always try to enhance their spectrophotometers by reducing the sources of errors to the lowest possible, ensures the repeatability and accuracy of the measurements, and minimize the test cost. In the following section, a demonstration for a successful method applied widely for some species that can reduce the cost sharply and make the spectrophotometer-like device available for virtually every interested person.

2- LED Based Spectrophotometers

Light Emitting Diodes or LEDs are semiconductor devices that produces light when a voltage applied over their terminals. They are designed to work at different wavelengths so that variant light colors can be obtained. Bi and Tri color LEDs are also available in which two or three semiconductor substrates were installed in one single package (e.g. RGB LEDs usually produces red, green, and blue colors). LEDs have many advantages since they are cheap, small, efficient, reliable, have low power consumption, and highly stable long life light sources.

LEDs are practically designed to produce light at a constant and narrow wavelength of approximately 25 nm wide. Since the absorbance bands for a particular medium were found to be typically in the 100 nm range, it is possible find a corresponding LED that falls within the molecular absorption range for a given medium.[14] This means that LEDs can be used as light sources instead of the expensive lamps used in conventional spectrophotometers for some particular wavelengths. Under such possibility many spectrophotometers based on LEDs as light sources were developed[15–21] and the first reported one was stated by Flaschka H., et al [22].

LED-based spectrophotometers makes the education of spectroscopy much easier since many of the components required to build it were available either on the shelf or can be afforded for inexpensive prices [23,24]. 3D printers, emerged with the broad capabilities of smart phones also have their impact on making simple and inexpensive spectrophotometers [25–30]. From the practical view, many inexpensive LED spectrophotometers can tackle money barriers in some specific purposes such as water analysis and monitoring[31–33], determination of metal ions[34,35], flow injection analysis (FIA)[36], and kinetics [37].

3- Experimental Work

3.1. Electronics and 3D Printed Parts

Like many conventional spectrophotometers, the proposed spectrophotometer has a dedicated LCD screen display, keypad to enter values and to select options, and a measuring chamber. The measuring chamber contains light source (RGB LED), cuvette holder, and the color/light intensity sensor. All the required parts were shown in Fig. **2** and the fully assembled device was shown in Fig. **3**.

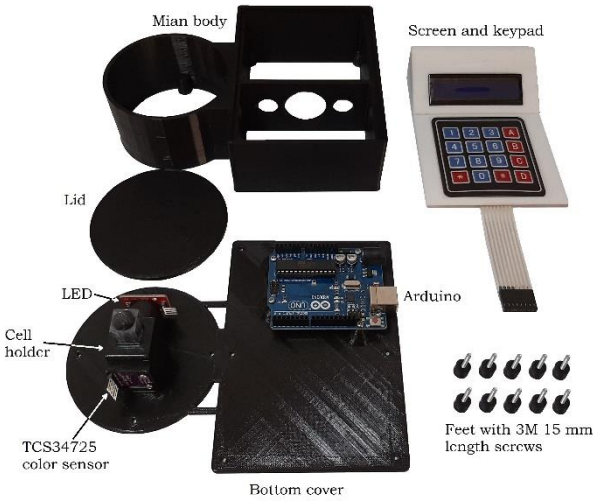

Fig. 2. Electronics and 3D printed parts

Fig. 3. Fully assembled spectrophotometer

The RGB LED model YSL-R1047CR4G3BW-F8 by Yun Sun LED Technology Co., Ltd. can source three different light colors of different wavelengths. According to the manufacturer, the LED can source red, green, and blue colors at peak wavelengths of 625, 520, and 468 nm. The LED have 10 mm diameter and a diffused domed top to ensure that light will propagate in all proposed directions with the same intensity. A current limiting resistor of 330 ohm was connected in series with each LED so that approximately 8 mA will be consumed by the red color LED and about 5 mA for both the green and blue LEDs. Current measurements were taken by connecting Lutron DM-9983G Smart Multimeter in series with each LED.

Color sensor TCS34725 by Adafruits was used as a photometer. It provides digital measurements for red, green, and blue color components and clear light as well. It also contains an IR blocking filter to overcome the interference of the uv light components in the incoming light. It can be supplied from 1.8-3.3 V and communicate with microcontrollers through the I2C bus protocol. The TCS34725 will measure the incident light components and measure its illuminance by:

$$\mathrm{I} = 0.2126R + 0.7152G + 0.0722B \quad (4)$$

The clear light component was cancelled since the designed spectrophotometer uses three color sources only (red, green, and blue). Then, the absorbance can be then measured via:

$$\mathrm{A} = log\left(\frac{I_0}{I}\right) \quad (5)$$

In which I_0 and I are the light intensity values for the blank and test sample respectively.

3D printed parts were designed using DesignSpark Mechanical free software and printed with Anycubic I3 Mega 3D printer. Black PLA filament (Torwell Technologies Co., Ltd) were used to print the bottom cover, main body, measuring chamber, lid, and electronics compartment to avoid any interference with external light sources. 4x4 matrix membrane Keypad (UrukTech Electronics) and 2x16 LCD display module (UrukTech Electronics) were attached over the electronics compartment cover, printed in white color to add some cosmetics to the device. Arduino Uno development board was used to control the operation of the spectrophotometer. Data received from the color sensor (through the I2C bus) will be represented in 16-bit for each component. This means that each color will have 2^{16} (65536) different values! Make the sensor so sensitive to any change in the color's intensity. Fig. **4** shows a schematic diagram for the circuit implemented in the design.

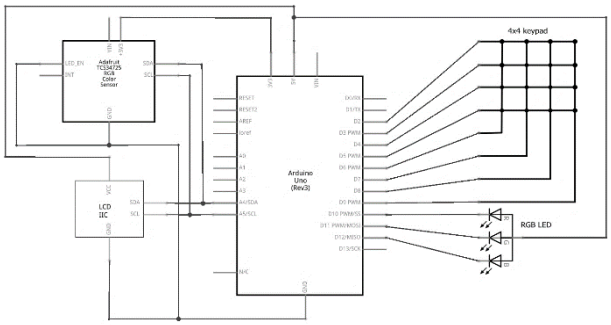

Fig. 4. Circuit schematic diagram

The design was aimed to be like conventional spectrophotometers but of course with a smaller size. The operator will be asked to select the color that its peak wavelength is close to the absorbance wavelength of the material to be measured. Thus, the operator will select one wavelength at a time depending to the chosen LED.

According to the calibration samples supplied, the device will calculate the best line that fits the calibration carve and uses its parameters to calculate the concentration of the unknown samples. The flow-chart and operation of the device was shown in Fig. **5**. To the best available LED-based spectrophotometers, the operator will have the opportunity to really simulate the work with real spectrophotometer with an easy-to-follow steps.

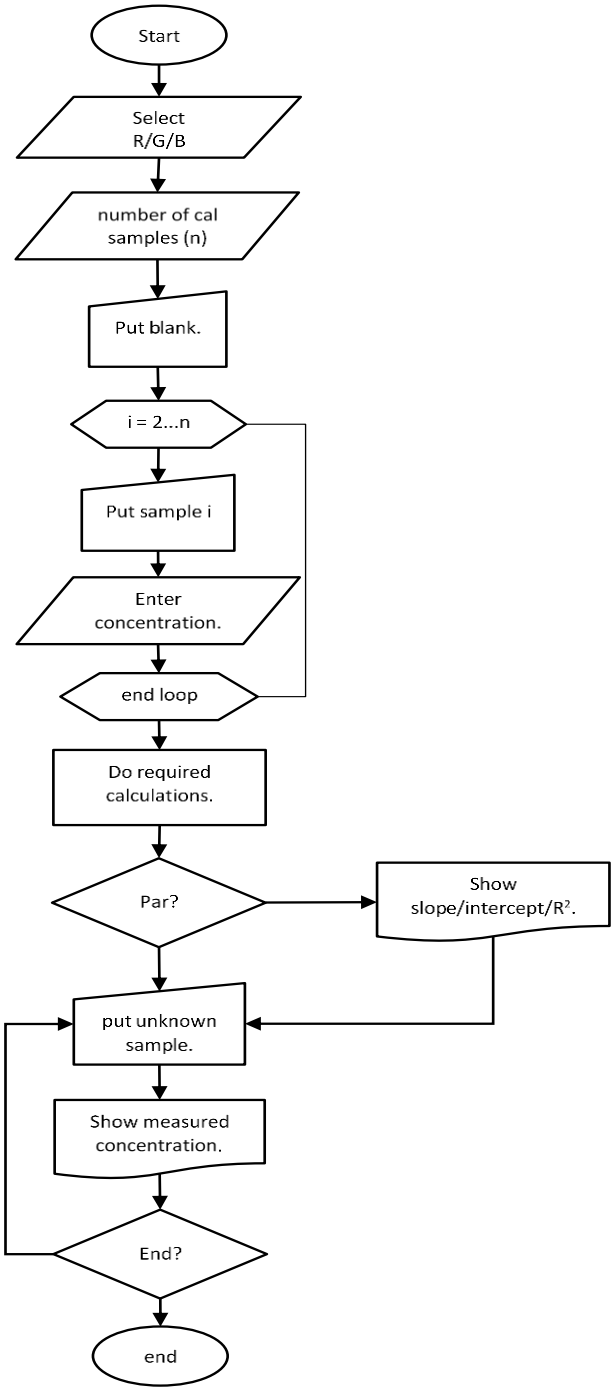

Fig. 5. Operation flowchart of the designed LED-based spectrophotometer

3.2. Preparation of Dye Solutions

A microscopy grade malachite green, methyl red and methyl orange (Thomas Baker, Mumbai, India) were used as test dye samples. Malachite green and methyl orange have peak absorbance at 617 and 464 nm respectively. Methyl red gives a peak absorbance at 520 nm in acidic media of pH 2.0. Methyl red solution were acidified using few drops hydrochloric acid. Five samples were prepared for each dye from stock solutions ranging from 0.5 up to 20 mg/l.

4- Results and Discussion

The LED based spectrophotometer was tested to measure the concentration of three different dyes. Generally, the LED color (i.e. either red of green, or blue) that its wavelength is close to the wavelength of maximum absorbance for the dye will be selected.

The measured value will be compared with that obtained by the Biotech Engineering UV-9200 spectrophotometer at maximum absorbance wavelength. Since standard cuvette were used in the measurements which have a 1 cm path length, this would make the measurements to be compared directly.

Fig. **6** shows the calibration curve for the measuring of Malachite Green dye. There is about 32% loss in sensitivity as compared to the measured value at absorbance peak of 617 nm by UV-9002 spectrophotometer.

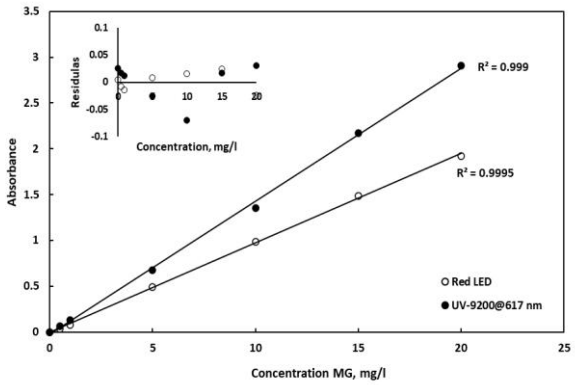

Fig. 6. Calibration curve for Malachite Green dye

Less sensitivity loss (about 7.5%) were obtained in the case of methyl red calibration measurements as seen in Fig. **7**.

This should be expected since the maximum absorbance for the methyl red matches the peak's emission band of the green LED. Finally, the sensitivity loss was found to be approximately 15% in the case of methyl orange dye calibration curve as shown in Fig. **8**.

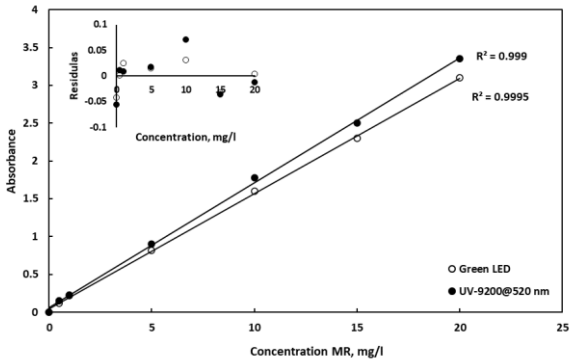

Fig. 7. Calibration curve for Methyl Red dye

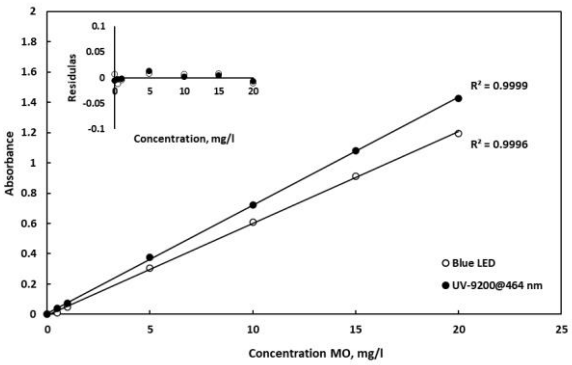

Fig. 8. Calibration curve for Methyl Orange dye

The general trend as can be seen from Fig. 6-8 is that there is always sensitivity loss in all measurements as compared to the conventional spectrophotometer measurements. Sensitivity loss could be minimized once the LED emission band is close to the maximum absorbance wavelength of the dyes. However, even in case of losing sensitivity, the accuracy of the measurement would not be affected. The R-squared values of the measurements were found to be mor than 0.99 in all over the measuring range for all dyes.

Linearities in Fig. 6-8 were further examined with the aid of residual plots that is embedded inside each figure. These plots reveal that the observed measurements fall with 0.02 absorbance unit for Methyl Orange and within 0.08 absorbance unit for both Malachite Green and Methyl Red. This confirms the high linearity of the relationship between the concentration and the absorbance as measured for both the LED based spectrophotometer and the UV-9200 spectrophotometer. The typical error[38], given by:

$$\text{typical error} = \frac{\sigma}{N^{1/2}} \quad (6)$$

where: σ is the standard deviation and N is the number of repeated measurements, based on repeating each measurement three times was found to be 0.0026, 0.0007, and 0.0023 absorbance unit for malachite green, methyl red, and methyl orange, respectively. This typical error falls within the precision 0.001 absorbance unit stated by the UV-9002 spectrophotometer or even butter. So, the precision of the LED based spectrophotometer is quite close to the conventional spectrophotometer standards.

5- Conclusions

A low-cost spectrophotometer was developed to tackle the commercial high-cost spectrophotometers. The developed spectrophotometer uses a simple RGB LED as source of light and color sensor as a photometer.

It can be used to measure the concentration of some widely used dyes confirming that their maximum absorbance wavelength should be close enough to the emission band width of the LED.

The device shows less sensitivity than the commercial spectrophotometer, but this should not affect the reliability of the measurements in all cases studied.

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قياس تراكيز الاصباغ باستخدام مطياف ضوئي واطئ الكلفة

بسيم حفيظ فاضل و ندى نعوم عبد الرزاق

قسم الهندسة الكيمياوية –كلية الهندسة – جامعة بغداد.

الخلاصة

تم تصميم مطياف ضوئي واطئ الكلفة لقياس تراكيز الاصباغ. تستعمل الاصباغ في تطبيقات مختلفة، لذا فان معرفة تراكيزها يكون امرا حيوياً لمختلف أنواع الدراسات. المطياف المقترح يستعمل العديد من المزايا التي يوفرها المطياف التجاري لكن بكلفة تصل الى اقل من خمسون دولاراً. الهدف من بناء المطياف هو توفير أداة تعليمية سهلة الاستعمال من قبل الطلبة والباحثين تساعد على فهم بعض خصائص العلوم الطيفية. أخضر الملكات، أحمر المثيل، وبرتقالي المثيل هي اصباغ انتخبت كنماذج لفحص جودة المطياف المقترح من ناحية الدقة، والتكرار، وحساسية القياس لغرض مقارنتها مع قياسات المطياف تجاري.

الكلمات الدالة: طباعة ثلاثية الأبعاد ، أردوينو ، مستشعر ألوان ، أصباغ ، مقياس طيف الضوء المرئي