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Characterization of excitation source LEDs and sensors without filters for measuring fluorescence in fluorescein and green leaf extract

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Abstract

This paper presents the characterization of excitation source LEDs and sensors without filters for measuring fluorescence in fluorescein and green leaf extract. For this purpose, eight light-emitting diodes (LEDs) were used with the following characteristics: one blue, one green, one red, one infrared, and four violets. The first four LEDs were used as sensors without filters to detect fluorescence induced by the other four violet LEDs in 11 samples of different fluorescein concentrations and in 14 samples of different dilutions of green leaf extract. The results show that infrared LEDs can detect the red emission of green leaf extract and red and infrared LEDs detect the fluorescence of fluorescein in concentrations of up to 1.8 μM. The developed system allows and facilitates teaching optical spectroscopy in basic education without incurring high costs.

Keywords*: chlorophyll, fluorescein, fluorescence, green leaf extract*, *light-emitting diode*

1. Introduction

Fluorometers are sensitive equipment that can classify biological materials of medical or agricultural importance by their fluorescent characteristics [1–9]. However, it is not easy to access this equipment due to its high cost and it is necessary to utilize them in an economical way starting from the research and characterization of materials obtained locally [5, 10, 11]. The design of low-cost fluorometers that improve the optical spectroscopy [12] of biological materials teaching in basic education institutions or in scientific research is a gap we must face [10, 13–15]. Currently, LED technology [16] as an excitation source has been replacing lasers because they are cheap, have low current consumption, and are easy to implement. All these characteristics make them ideal for the development of fluorometers in schools, colleges, and universities. A proof of this is that in 2013, a fluorometer to measure chlorophyll was built for less than $145 by using a 425 nm LED as an excitation source and a red Roscolux filter in front of a photodiode receiver (PD) [17].

A slightly more sophisticated device was designed by Puiu and collaborators in 2015, they developed a submersible to measure chlorophyll-a, oil, and protein material (tyrosine and tryptophan) in natural waters by using ultraviolet (UV) LEDs at 280 nm and blue LEDs at 450 nm and a USB2000 + spectrometer as the detection system [18]. LEDs are not only used as an excitation source but also as fluorescence sensors. The use of LEDs as sensors is not a new technique; in 1992, a chip with an array of six LEDs was developed and implemented in a solar photometer to measure atmospheric turbidity and precipitable water [19]. In 2005, Acharya developed a solar photometer to measure variations of optical depth of the atmosphere using three LEDs (red, yellow and green) as a detection system and demonstrated that the spectral response of LEDs is oriented toward wavelengths of greater power [20].

Lau and collaborators in 2006 developed an optical device for colorimetric analysis based on a light-emitting diode (LED) as the detector; the equipment was validated through

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absorbance measurement with Bromocresol Green and then used to detect cadmium(II) and lead (II) in water [21]. O'Toole and collaborators in 2007, by Malachite Green method used for the determination of phosphate, implemented a detection system with a pair of LEDs: one as the excitation source and the other for colorimetric detection [22]. A multi-color RGB LED (emitting red at 625 nm, green at 527 nm, and blue at 460 nm) has been implemented to work simultaneously as a light emitter for excitation and luminescence receptor in phosphor materials (500–650 nm) [23]. In 2012, Pokrzywnicka and collaborators developed optoelectronic flow detectors in miniature to determine photometrically and fluorometrically proteins such as albumin and globulins [24]. Fiedoruk and collaborators in 2015 were able to detect phosphorus in soils and calcium and phosphorus in human sera by using external fluorophores as markers, inducing fluorescence with two green LEDs of 525 nm, and then detecting the fluorescence with a red LED of 650 nm [25, 26]. In 2018, Fiedoruk et al. performed the photometric determination of hemoglobin in human blood as well as fluorometric determination of quinine in tonics and calcium ions in mineral waters using these dual LED systems [27].

All these works report the results of LED applications as excitation sources and light sensors; however, none makes a detailed and simple characterization of the requirements for its implementation, which as well as the fluorescence measurement of fluorescein and green leaf extract (total chlorophyll), is the purpose of this work. Eight LEDs (one blue, one green, one red, one infrared, and four violets) are used in the test, where the first four are used as sensors without optical filters and the last four are used as the excitation source. To show this, section 2 presents the materials and methods, section 3 describes the results and analysis, and section 4 includes the conclusions.

2. Materials and Methods

2.1. Characterization of LEDs

Four LEDs of easy acquisition were used as sensors: infrared LED (IrLED), red LED (rLED), green LED (gLED), and blue LED (bLED), all with transparent 5 mm encapsulation. Four violet LEDs (vLEDs) were used as the excitation source. All LEDs were assembled in a cuvette holder as shown in Figure 1 and placed in a black box to avoid interference by external light. The emission spectra of the eight LEDs were obtained with a FLAME-S miniature spectrometer from Ocean Optics at a diode current of 15 mA. Table 1 reports peak wavelengths and bandwidths obtained for five types of LEDs (the four vLEDs presented the same spectrum).

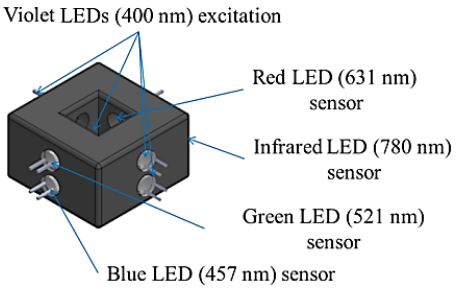


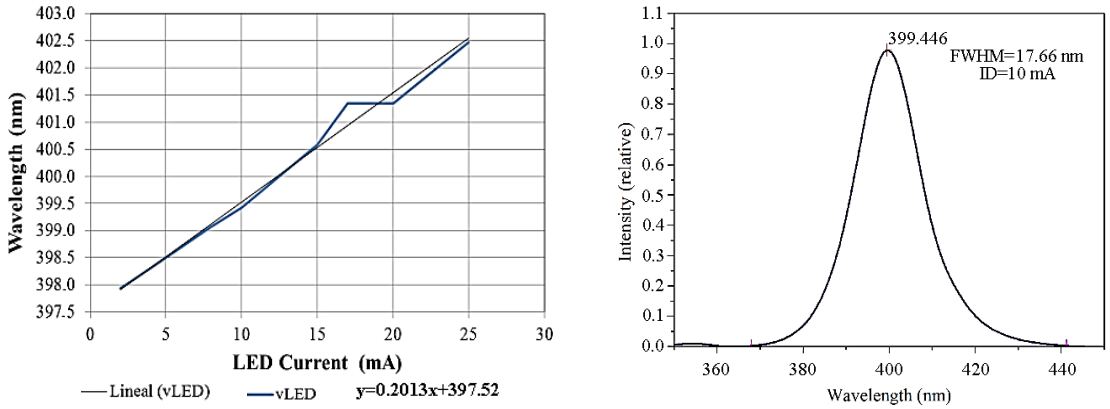
Figure 1. Assembly of LEDs in cuvette holder

Table 1. Emission Spectra Details of Four Sensor LEDs and Violet LED Used as Excitation Sources

Type of LED	Wavelength Peak (nm)	Bandwidth (nm)
Infrared	780	33
Red	631	21
Green	521	37
Blue	457	23
Violet	400	18

To guarantee the emission spectrum of vLED, the wavelength shift was characterized at 25°C with respect to current changes in the LED using a Fluke 87VC multimeter. Figure 2 (a) shows the results obtained and linear regression; it is possible to see that between 10 mA and 15 mA, the emission is maintained at 400±0.5 nm. Figure 2 (b) shows the emission spectrum of the vLED at a current of 10 mA, which presents a width at half-height (FWHM) of 17.66 nm and maximum emission at 399.45 nm.

Knowing the current and emission wavelength of the violet LED, irradiance was measured according to voltage. For this, the current was adjusted to 15 mA and a pulse width modulation (PWM) was made with an Arduino Pro Mini with ATmega328 microcontroller. Irradiance was measured with a DeltaOhm HD 2302.0 radiometer. Figure 3 shows the near linearity of average irradiance with respect to vLED voltage (linear regression R^2=0.9999).



(a) (b)

Figure 2. (a) Changes in vLED wavelength with changes in current and (b) vLED emission spectrum

Given the above data, excitation LEDs were configured as follows: LED current 15 mA, central wavelength 400.582 nm, and LED voltage 3.2 V, corresponding to an irradiance of 7.4 W/m^2. Voltages generated by LEDs used as sensors were not amplified; they were read directly by the analog-to-digital converter (ADC) of the PIC16F1937 microcontroller from Microchip Inc., sent by Bluetooth to a cell phone (Free apps: BT Simple Terminal, for Android), and processed in OriginPro 8. The irradiance of 400 nm LEDs can be calculated with (1) by applying a PWM to the LED, where *I* is the irradiance and *V* is the voltage on the LED:

$$I = 2306.6 * V + 2.948 \quad (1)$$

LEDs can have a spectral response thanks to the photoelectric effect, whose energy (E) can be written as in (2) [28], where h is Planck's constant, υ is the minimum frequency of radiation (so there is a photoelectric effect), ω is the work function, and eV_0 is the maximum kinetic energy of electrons.

$$E = h\upsilon = eV_0 - \omega \quad (2)$$

In (1), it is observed that the energy of the incident photon is directly proportional to the radiation frequency; therefore, lower-energy photons (a red LED of 630 nm, for example) do not induce a photocurrent in a blue LED of 457 nm.

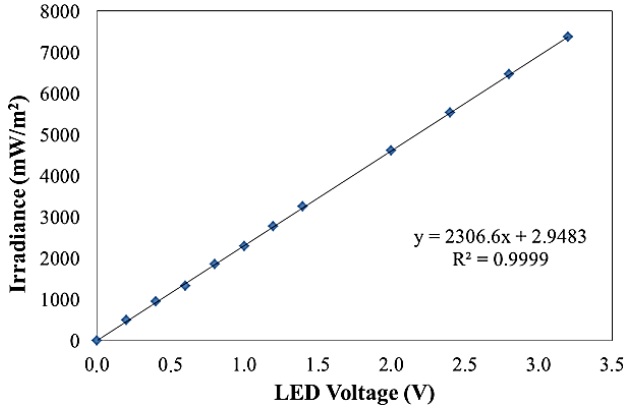


Figure 3. Average irradiance of vLED according to the voltage applied with a PWM

2.2. Extraction of green leaves

Green leaf extract was obtained from 0.5 g of green leaves in 10 mL of 96% ethanol (CH_3CH_2OH) that was macerated and centrifuged (ROTOFIX 32A Hettich centrifuge) at 4,500 rpm for five minutes. The supernatant was filtered using filter paper (MUNKTELL grade 391) with particle retention of 2–3 µm; this sample was diluted to 1:8, 192 to yield a total of 14 samples. Chlorophyll concentration was not determined because the purpose was to determine LED sensitivity in the detection of fluorescent radiation.

2.3. Preparation of fluorescein

For fluorescein, 11 samples of concentrations 602, 301, 150.5, 75.3, 37.6, 18.8, 10, 7.2, 5.4, and 1.8 µM were prepared in a solution of monopotassium phosphate (KH_2PO_4) and dipotassium phosphate (K_2HPO_4) with a pH of 7.6. The fluorescence spectra of fluorescein as shown in Figure 4 and green leaf extract as shown in Figure 5 were obtained with the same spectrometer used to characterize the LEDs, which are in the green and red regions, respectively. At the beginning of each measurement, readings of background noise and sample blanks were obtained and subtracted from sample readings.

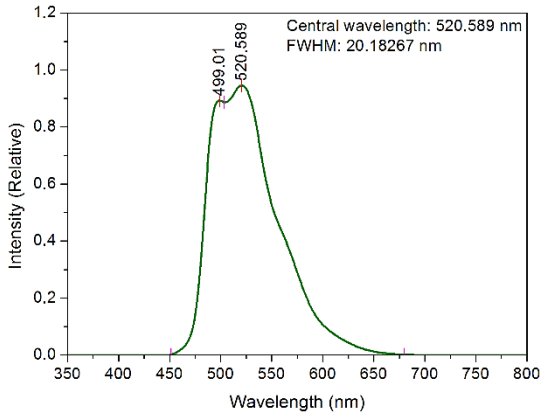


Figure 4. Fluorescent emission spectrum of fluorescein

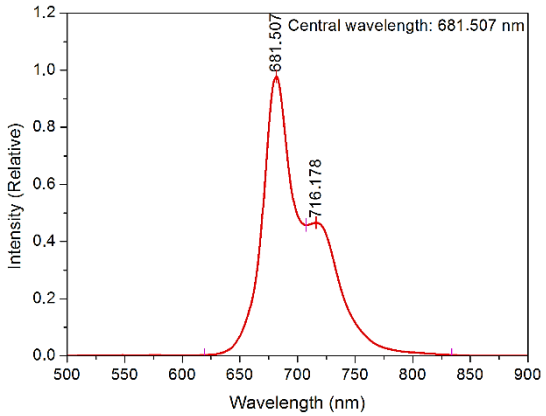


Figure 5. Fluorescent emission spectrum of green leaf extract

3. Results and Analysis

Figure 6 shows four channels that measure the response of LEDs (x axis) to the fluorescence of different dilutions of green leaf extract. It is clear that the LED that gave a response with greater discrimination with respect to different dilutions of sample was IrLED, whereas rLED, gLED, and bLED produced overlapping responses. Figure 7 shows the response of IrLED to different dilutions of green leaf extract, detecting dilutions of 1:8, 192 with an IrLED voltage of 0.06 V without amplification. The voltage dropped below dilutions of 1:4, which could be related to the internal filter effect due to high chlorophyll concentrations in the samples with dilutions 1:1 and 1:2.

Responses of the four LEDs to fluorescein are represented in Figure 8. This result shows that LEDs that discriminate the best at different concentrations were rLED and IrLED; the other two LEDs provided an overlapping response. Figure 9 shows the responses of RLED and IrLED for different concentrations of fluorescein. These data show that at concentrations of 1.8 µM, RLED gives a voltage 0.2 V and IrLED gives a voltage of 0.038 V with no amplification.

Figure 10 shows that LEDs responding to different fluorescence have longer emission wavelengths. That is why the LEDs, bLED, gLED, and rLED do not respond to the fluorescence of green leaf extract (emission at 680 nm); however, IrLED, which is the least energetic, does. On the other hand, for the fluorescence of fluorescein (emission at 520 nm), rLED and IrLED respond, whereas bLED and gLED that corroborate the shift between the emission and the spectral response of the LEDs do not [20]. Pseudo-replicates of the 11 fluorescein solutions were made two days later to examine the stability of the readings. Figure 11 shows the high reproducibility in the fluorescence detection of LEDs.

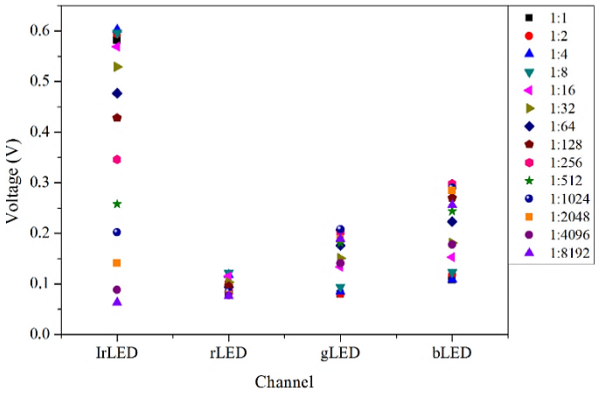


Figure 6. Responses of four LEDs to red fluorescence of green leaf extract

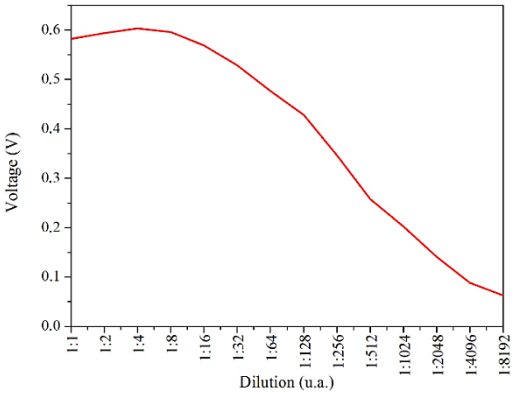


Figure 7. Response of IrLED to different dilutions of green leaf extract

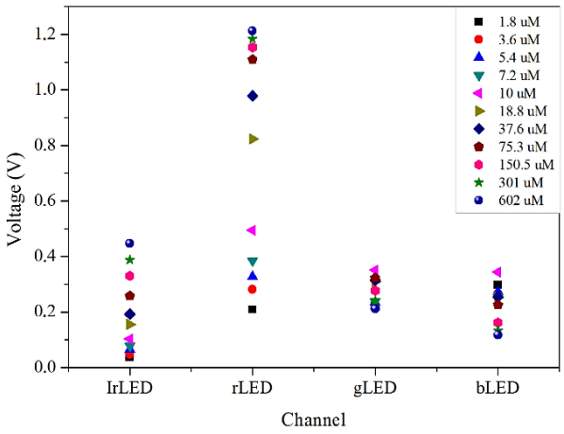


Figure 8. Responses of four LEDs to green fluorescence of different fluorescein concentrations

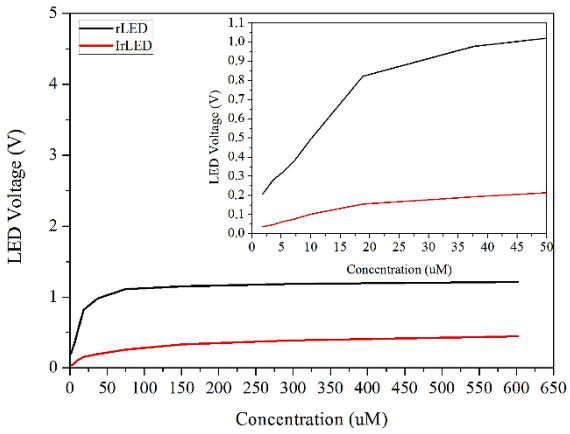


Figure 9. Responses of r LED and IrLED to different dilutions of fluorescein

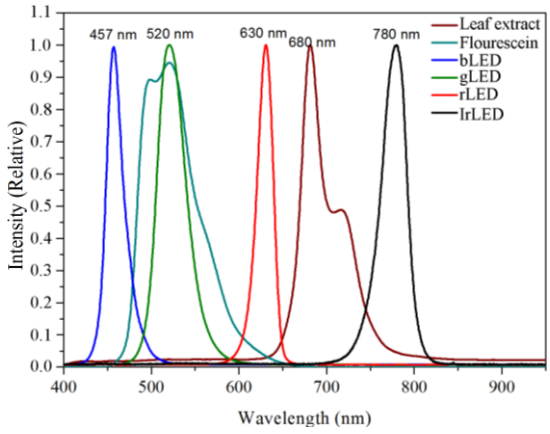


Figure 10. Fluorescent emission spectra of samples under study and responding LEDs

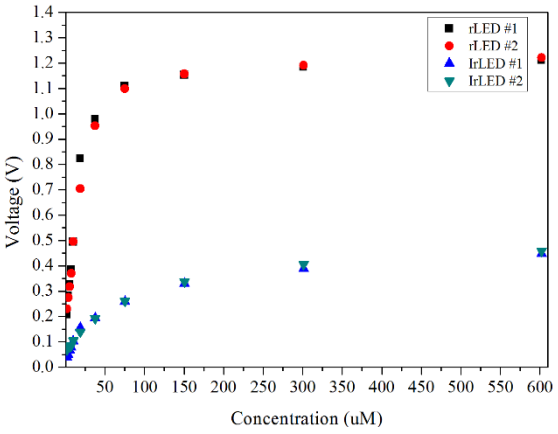


Figure 11. Responses of red and far-red LEDS to the repetition of measurements for fluorescein samples

4. Conclusion

This paper presented the characterization of excitation source LEDs and sensors without filters for measuring fluorescence in fluorescein and green leaf extract. The results show that the more energetic the LED (shorter wavelength), the narrower its spectral response, making it a built-in bandpass filter. This allows the use of LED sensors, which is an inexpensive option for the development of fluorescence measuring equipment. A 780 nm emission LED can be used as a fluorescence sensor of green leaf extract whose fluorescence could be related to the total chlorophyll present in the leaf. LEDs with 631 nm (red) and 780 nm (IR) emissions can be used as sensors of fluorescence for fluorescein, and with a suitable calibration to quantify it. To achieve guaranteed readings at low concentrations, it is important to insert an adjustable gain amplifier. It is also important to guarantee the LED current to keep the wavelength stable when used as an excitation light source. A high concentration of fluorophores will have low spectral response of LEDs due to the internal filter effect from the sample. Finally, the irradiance of the LEDs can be calculated by using the equation that considers only the voltage applied with a PWM.

Acknowledgments

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