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A palm-sized wireless device for colorimetric nitrite determination in water

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ABSTRACT

In this work a palm-sized, affordable, and wireless device is presented for colorimetric determination of nitrite in water, based on the Griess method. A RGB sensor APDS 9960 was used, as a detector, to determine the amount of nitrite in water and the color intensity of the dye produced by the reaction between nitrite and Griess reagent was detected. The detection system consisted of a green LED (λ_{max} = 530 nm) used as light source and the signals were monitored by selecting the green channel. An ESP32 board was used for signal processing and this ESP board includes a housing having space for receiving a lithium-ion battery, used for the power source of the circuit. Also, this boards act like a router which was employed to transfer data between the devices. A detection limit of 2 μgL^{-1} (3σ) was achieved by the proposed procedure with a relative standard deviation value varied from 1.6 to 2.5 for intra-day (and from 2.4 to 2.7 for inter-day precision, when a Green and White LEDs were used, respectively. With a low reagent demand of 20 µL, the proposed device is environmentally friendly, ideal for *in situ* determination and the resulting battery life of a 7-hours is feasibly for nitrite monitoring in different water samples. A comparison between the presented procedure employing a white and a green LEDs, used as light source, was conducted for nitrite determination and the results were compared with those obtained by UV–vis. The proposed procedure employing the green LED was more sensitive and provides greater accuracy.

1. Introduction

Colorimetric determinations based on digital images has increasingly employed the smartphone camera as a system of detection [1]. Quantitative determinations are processed after acquisition of an image obtained by the built-in smartphone camera [2]. Using a specific image processing software, sample color is decomposed and quantified to its red (R), green (G) and blue (B) components [2]. It should be emphasized that the light source conditions, camera characteristics, and their distance to the sample may keep constant to reach a reliable precision and accuracy [3]. Some advantages of smartphone-based digital imaging include simplicity of use, low-cost acquisition, portable and wireless connectivity via Bluetooth or Wi-Fi, resulting in friendly analytical procedures, ideal for environmental monitoring [3–5].

Similarly, the use of RGB sensors has been reported for the determination of nickel [6], cyanide [7] e, lead [8] and iron [9]. Besides, RGB sensors interfacing through with microcontrollers, are becoming the core engine for sensor-based analytical systems [10]. The ability to create a core platform and implement only low-cost software for analytical applications has driven the rise in the availability and diversity of development boards for analytical purposes [10,11]. Wireless data communication may be performed by the integration of a Bluetooth or Wi-Fi module [12]. Generally, wireless based modules are cheap and easy to interface with microcontroller and optical sensors, showing a low power consumption. Furthermore, wireless communications allow devices to remain connected to a network, one of the requirements to generate the Internet of Things (IoT) devices [13].

The development of a sustainable, portable, and cost-effective approach to chemical water monitoring in the natural environment waters is highly important, since it can be indicated, in real time, the quality of water for ecosystem health. Portable analytical devices based on the concept of Internet of Things are becoming of considerable concern to many countries worldwide nowadays, contributing to the advance of *in situ* analysis. Recent enhancement between IoT and analytical chemistry have catalyzed considerable progress in remote environmental monitoring. As a result, the ability to determination chemical properties of water quality at adequate temporal and spatial scales has considerably improved [14]. These advances demonstrate the potential to improve overall outcome statistical and chemical modeling in monitoring of water quality trends. In addition, they have greatly

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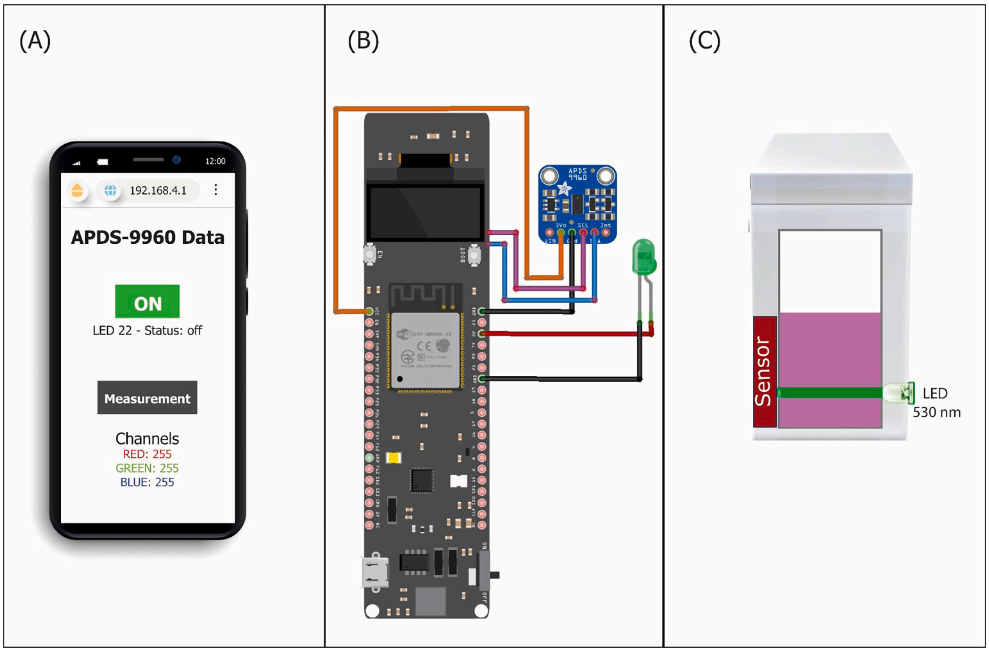

Fig. 1. Block scheme of the portable wireless device for nitrite determination. (A) Smartphone graphical interface. (B) ESP32 board, sensor, and LED connections. (C) Measuring compartment.

enhanced real-time detection, recognized as a critical need for early warning systems to properly identify, as beforehand as possible, those species that might potentially be hazardous. In this sense, relevant contributions have been made on fields such as point-of-care and *in vivo* detection, food control and environmental analysis [5].

Nitrite (NO_2^-) is a naturally occurring anion in fresh waters and contamination of water with this ion usually comes from use of chemical nitrogenous fertilizers, food additives and atmospheric deposition of nitrogen compounds [15]. Nitrogen species present in the air and originating from natural and anthropogenic sources (industrial and agriculture activities) after the reactions with water come back to the earth surface in the form of acid rains [16]. The most widely colorimetric methods used in routine laboratories for nitrite determination are based on Griess reaction. This method is based on the diazotization-coupling reaction of nitrite by sulfanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride (NED) in acid media. The final product acquires a rose color with maximum absorption at 540 nm.

In the present work, a portable and wireless device, based on Griess method, for the determination of nitrite in water was designed and built. The device is based on colorimetric technique, particularly in the use of a RGB sensor as detector and a green LED was selected as light source. Compared to the white LED, commonly used as light source in others RGB sensors, this device has advantages such as better accuracy and sensitivity. Subsequently, the developed RGB sensor was applied for nitrite determination. To our knowledge, no one has reported application of a complementary color as light source for colorimetric determination based on color sensor analysis.

2. Experimental

2.1. Chemicals, reagents, and samples

Nitrite reference solutions were prepared by dilution of a 1000 mg L^{-1} (NO_2^-), prepared using sodium nitrite and standardized by permanganometry. Griess reagent was prepared with 10 % (v/v) phosphoric acid, 80 $mmolL^{-1}$ sulfanilamide (Vetec, Rio de Janeiro, Brazil) and 40 $mmolL^{-1}$ (m/v) N-(1-naphthyl)ethylenediamine dihydrochloride (Sigma-Aldrich, German). Reagents were stored in amber glass storage bottles and maintained at 4 °C.

2.2. Apparatus

The LEDs emission spectrum were obtained with a CCD spectrophotometer (USB2000, Ocean Optics, Dunedin, FL, USA). RGB color sensor response was evaluated employing a spectrophotometer (FEMTO 600 s spectrophotometer), accordingly previously reported [9]. A digital smartphone camera (TECNO Spark4, Android 9) was used for sample image acquisition while ImageJ software as used for image processing and analysis.

A RGB, proximity, light and gesture sensor, APDS-9960 (Sparkfun) [17] was used for signal acquisition. With small dimensions, L 3.94 × W 2.36 × H 1.35 mm, the sensor is based on a photodiode array, comprises a 4 × 3 array of Red Green, Blue and Clear. When connected to ESP32 board, the sensor returns the values of red, blue, green, and clear light into an 8-bit system. This device was connected to an OLED Wi-Fi Bluetooth ESP32 board (Filipeflop, Brazil) [18] was used as a central processing unit of the detector to receive and execute the commands. This ESP board includes a housing having space for receiving the 18,650 lithium-ion polymer battery (3.7 V, 4800 mAh), used for the power source of the circuit. A charging system engaged in the board was used to charge the battery via Micro USB charge cable, compatible with smartphones. The ESP32 board was programmed using Arduino IDE environmental and the RGB data were obtained through using the software and libraries provided by the manufacturer. A sketch designed on Color Sensor [17] was used to provide output data for each channel.

A 3D printed cuvette white adapter was manufactured to allocate a semi-micro polystyrene cuvette (1 cm path length, and 1.5 mL), the RGB sensor and land a super bright green LED (5 mm). The LED has a peak emission wavelength of 530 nm, connected to one of the GND and 3.3 V ESP32 pins, and positioned at 180° in relationship to the sensor, aligned with the color detector.

The ESP32 board was set as access point by Wi-Fi network and connected to the smartphone. A HTML and C/C++ based software was developed to control the measurements, display the results in real-time, and transmit them to the smartphone, by connection via web browser at address httpp://192.168.4.1. With the software, the user could

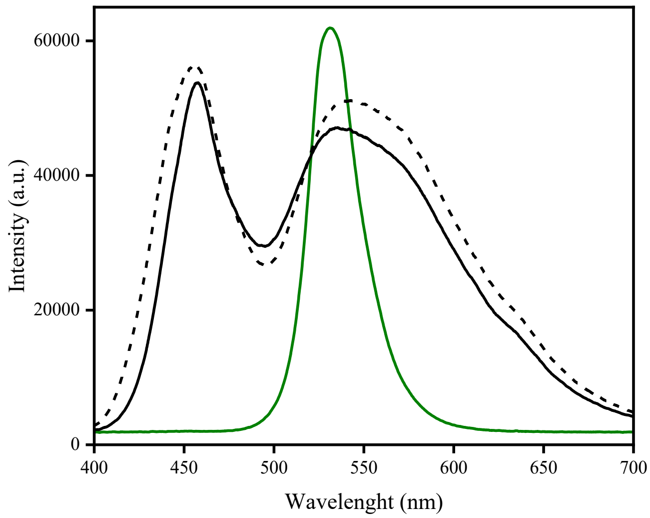

Fig. 2. Emission spectrum of LEDs evaluated as light source for nitrite determination. (—) green ultra-high brightness; (—) white ultra-high brightness and (− − −) white surface mounted diode (SMD).

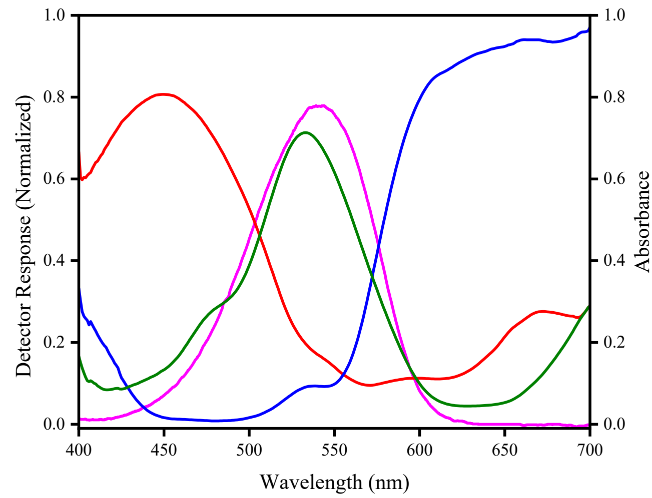

Fig. 3. Overlap of absorption spectrum of the azo dye produced by the reaction between nitrite and Griess reagent (—) and effective sensor response for the R (—), G (—) and B (—) channels.

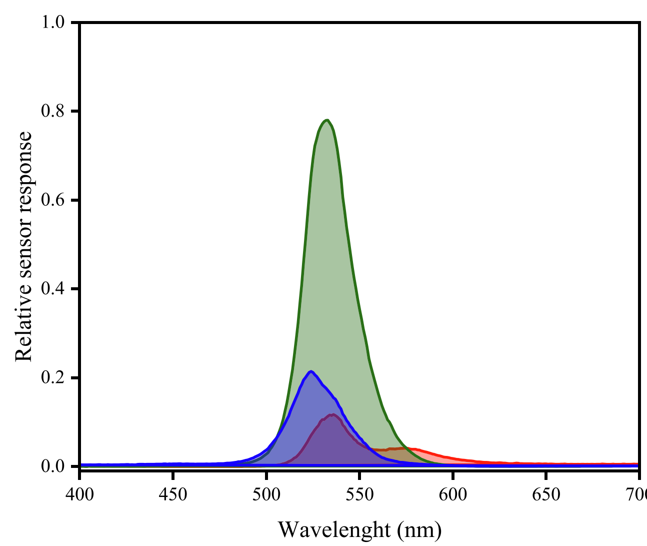

Fig. 4. Effective capability of the RGB sensor spectrum for the R (—), G (—) and B (—) channels using the green ultra-high brightness as light source.

conveniently select the proper instrumental parameters, turn on or off the external green LED in the graphical interface, and make the measurements. All measurements of the three channels are registered on the display, until clicking to perform a new measurement. Analytical signal measured by the device is based on absorbance. The sensor makes the RGB scale adjustments for reference and sample measurements. The absorbance was calculated according to the equation:

Absorbance = -log I/I_0.

Whereby I is the signal for the sample, I_0 is the blank value of the green channel. A block scheme of the portable device is showed in the Fig. 1. A prototype photography is showed in the Supplementary material (S1). The circuit schematics used in this photometer is show in Supplementary material (S2).

2.3. Procedure

The determination of nitrite was performed as follows: 20 μL of the Griess reagent was transferred directly to the test tubes containing 500 μL of the sample or nitrite reference solutions and mixed manually. The solution was transferred into a semi-micro polystyrene cuvette and submitted to the measurements after 10 min.

Different natural water samples were analyzed in this work for nitrite determination. Tap water and rainwater were collected in the city of Uberlândia. River waters were collected in Rio Uberabinha (Uberlândia, State of Minas Gerais). Freshwater was obtained from local market. Water samples were stored at 4 °C and analyzed after filtration.

3. Results and discussion

In this work a RGB color sensor APDS 9960 (Sparkfun) was used for quantitative determination of nitrite in water samples based on Griess reaction. Usually, RGB sensors come as standard with a built-in LED white LEDs providing favorable light source conditions for colorimetric detection, as reported previously [6–9] Unlike others RGB sensors, the APDS 9960 sensor has not a built-in LED, it is necessary to insert an external light source. At first it may seem a disadvantage, the addition of an external light source makes it possible to explore different types of LED and their respective positions settings, in relationship with the detector. A white surface mounted diode (SMD) and two ultra-high brightness LEDs, white and green are selected for this purpose. The SMD LED chosen is similar to that used in TCS 34725 color sensor [9,19]. The ultra-high brightness white LED is similar those that are used in TCS230 [8] and TCS3200 [7] color sensors. Consequently, a green LED was chosen as the light source for the sensor design by the fact that is a complementary color of the dye produced by the reaction between nitrite and Griess reagent. All used LEDs were characterized, and their emission spectrum was measured by the spectrometer and is presented in Fig. 2. The white LEDs are almost identical and exhibit a full spectrum emission in the visible wavelength range from 400 to 700 nm.

3.1. Spectral characteristics

The sensor response was evaluated as a function of wavelength. A commercial spectrophotometer was used as a monochromatic light source sand the s and the radiation from the light source of the spectrophotometer was incident directly on the sensor. The Fig. 3 illustrates the RGB responses for each wavelength evaluated (1 nm increment), showed together with the absorption spectrum of the azo dye produced by the reaction between nitrite and Griess reagent. The azo dye produced had an absorption peak at 540 nm, which correspond to the G channel sensor response. The green LED emission spectrum overlapped and contrasted with the azo dye UV–vis absorption spectrum. The

Table 1
Comparison of the prosed procedure employing APDS9960 RGB, TCS34725 RGB sensors and a conventional spectrophotometer.

Detector	LED	Curve equation	Linear range (μgL^{-1})	RSD [a] (%)	R^2	LOD[b] (μgL^{-1})
Sparkfun APDS 9960	White	$y = 0.543\ C_{NO_2^-} + 5.5 \times 10^{-3}$	30–1200	0.57	0.998	10
	Green	$y = 0.796\ C_{NO_2^-} + 6.9 \times 10^{-3}$	6–400	0.45	0.997	2
TCS34725	White	$y = 0.431\ C_{NO_2^-} + 0.011$	30–1200	0.98	0.997	9
	Green	$y = 0.549\ C_{NO_2^-} + 3.4 \times 10^{-2}$	20–400	1.07	0.998	7
Smartphone	White	$y = 0.450\ C_{NO_2^-} + 2.6 \times 10^{-2}$	60 –1200	0.76	0.998	20
Spectrophotometer		$y = 0.715\ C_{NO_2^-} + 2.3 \times 10^{-3}$	10–1000	0.41	0.999	4

a–n = 10.
b–Limit of detection.

effective sensor response was obtained in the Fig. 4, corresponding to the result of the multiplication of the green LED emission spectrum and the sensor response.

3.2. RGB system performance

To study the effect of light intensity on RGB values, two other sources yielding different light intensities were compared. The analytical performance, obtained when the white and green ultra-high brightness LEDs were used for sample light source, were estimated for this purpose. Analytical characteristics obtained with the RGB sensor coupled with a green LED were compared with those obtained employing the white LED, operating under the same conditions of current. Sensitivity obtained using the green LED was about 45 % higher than that achieved with the white LED.

Therefore, additional studies have been conducted employing the TCS34725 RGB sensor at same instrumental conditions. In the case of TCS34725 sensor, the SMD white built-in LED are positioned at approximately 5° in relationship to the detector. To evaluate the effect of the light source position settings, an identical SMD LED was used positioned at 180° in relationship to the sensor. The results obtained by both LED position were in agreement (differences lower than 8 %), indicating that the sensor's measure capability occurs at the cuvette interface, and both strategies were sufficient to promote adequate lighting. Other analytical characteristics were estimated between RGB sensors and light source. Similar behavior was observed between the sensors, and high sensitivity was obtained when the green LED was used as a light source. However, the APDS9960 RGB color showed higher sensitivity and lower detection limit. Finally, the analytical features were obtained for each sensor, both illuminated with a white and a green LED and compare with those obtained employing smartphone camera and a conventional spectrophotometer (Table 1). Similar responses were observed among RGB sensor, spectrophotometric measurements, and smartphone camera. Under optimal conditions, the proposed procedure employing APDS9960 RGB color and a green LED as a light source showed the lower detection limit, 2 $\mu g\ L^{-1}$.

The precision of the developed method was evaluated as intraday and interday precision, determined by analyzing 84 replicates (every 5 min), on the same and five different days, respectively. The RSD values varied from 1.6 to 2.5 for intra-day (and from 2.4 to 2.7 for inter-day precision, when a Green and White LEDs were used, respectively. The low RSD values of intra-day and inter-day indicated that the developed method has a high precision. Table 2 shows a comparison between some characteristics of the proposed method, and those of different colorimetric procedures reported in the literature involving nitrite determination. It can be concluded from Table 2 that the proposed procedure showed analytical features comparable with most of the reported procedures and more than a few of the reported methods have a lightly lower detection limit [19–36]. Analytical features are comparable or better than that achieved with previously reported Griess-based procedures [19–29].

In order to assess the applicability of the proposed procedure, the

Table 2
Comparison of the present procedure with other reported colorimetric procedures for nitrite determination.

Method/detection	Linear range (µg/L)	LOD (µg/L)	RSD (%)	Ref
Griess-Based Methods				
RGB sensor with a green LED	6–400	2	0.45	This method
Digital image (Smartphone)	50–10000	30	5.8	[21]
Digital image (Smartphone)	90–1800	30	<1	[22]
Digital image (Scanner)	–	55	5.9	[23]
Microfluidic and Spectrophotometer	–	34	1.9	[24]
Microfluidic fiber optics	20–1800	7	0.37	[25]
Sensor and Spectrophotometer	10–200	10	11.7	[26]
Spectrophotometer	40–1100	3.79	–	[27]
Spectrophotometer	70–5000	7	4.5	[28]
Spectrophotometer	0–3000	48	0.88	[29]
Spectrophotometer	10–420	2.2	0.46	[30]
Spectrophotometer	100 –1000	8	0.8	[31]
Other colorimetrics Methods				
Reaction with Iodide in acid medium	100–2000	25	0.6	[31]
Nitrite reaction with Zein biopolymer nanoparticles	5 –1000	2.3	–	[32]
Oxidation-reduction by iodide in acid medium	8–120	6	3.4	[33]
Redox reaction with $KBrO_3$ and crystal violet in acid medium	30–300	0.3	<2	[34]
N-Phenyl-p-phenylenediamine reaction with N, N–dimethylaniline	2–100	0.6	2.4	[35]
Reaction with barbituric acid in acidic medium	0–322	–	0.5	[36]
Reaction with 4-Amino-5-hydroxynaphthalene-2,7-sulfonic acid monosodium salt	100–1600	6.9	0.46	[37]
Reaction with dapsone (DAP) and α-naphthol	50–800	1.12	0.0017	[38]
Reaction with dapsone (DAP) and 4-Amino-5-hydroxynaphthalene-2,7-sulfonic acid monosodium salt	200–1400	82	0.1687	

Table 3
Nitrite determination in water samples (mean values and uncertainties, n = 3). Concentrations in µg/L.

Samples	Sparkfun		Smartphone	UV–vis [b]
	White	**Green**		
Rainwater 1	31.9 ± 4.9	32.8 ± 4.4	< LQ	34.8 ± 2.9
Rainwater 2	49.9 ± 4.1	39.2 ± 3.5	< LQ	40.8 ± 2.1
Rainwater 3	108.8 ± 2.5	99.8 ± 1.7	116.6 ± 3.7	102.8 ± 2.8
Rainwater 4	113.1 ± 4.2	107.1 ± 2.0	114.5 ± 6.4	107.5 ± 1.6
Rainwater 5	40.1 ± 2.0	37.2 ± 4.2	< LQ	39.0 ± 1.6
River water 1	62.3 ± 2.9	56.4 ± 2.1	62.6 ± 3.5	56.2 ± 2.1
River water 2	170.0 ± 2.0	172.1 ± 1.0	172.8 ± 3.9	173.6 ± 3.2
Freshwater	< LQ	7.3 ± 1.0	< LQ	7.7 ± 2.4
Tap water	< LQ	8.9 ± 0.7	< LQ	10.1 ± 1.6

Table 4
Spike test for nitrite determination in selected water samples (mean values and uncertainties, n = 3).

Waters samples	NO^{2-} add (μg L^{-1})	Recovery %) White	Green
Rainwater 2	25	94.3 ± 2.2	101.3 ± 1.3
	50	104.1 ± 1.1	93.8 ± 0.7
Rainwater 4	25	83.6 ± 1.1	96.9 ± 0,5
	50	97.0 ± 0.5	87.1 ± 1.6
Rainwater 5	25	99.3 ± 2.3	99.2 ± 1,6
	50	100.8 ± 1.8	101.9 ± 1,8
River water 2	25	96.3 ± 0.9	94.5 ± 0.4

optimized method was employed for the determination of trace amounts of nitrite in rain, river, fresh and tap water samples and the results are listed in Table 3. A comparison between the presented procedure, employing two different LEDs as light source — white and green — was conducted for nitrite determination, and the results were compared with those obtained by UV–vis [20] and a smartphone camera. When the white LED was selected for as light source, a positive interference was observed for the Rain (2, 3 and 4) and River 1 water samples. These interferences can be attributed to spectrum overlaps among the RGB sensor response, observed especially for the green channel, according to what was observed in previously work for iron determination [9]. The nitrite concentrations in fresh and tap water samples were below the detection limit of the ion obtained with white LED, which indicated that the proposed sensor employing the green LED was more sensitive and provide greater accuracy. In all other cases, the results agreed with those obtained by the UV–vis at a 95 % confidence level. With the ESP board used in this work, the self-contained rechargeable running up to 7 h on battery.

A recovery study was performed to assess the occurrence of matrix effects. Water samples were spiked at 25 and 50 μg L^{-1}, using a green and white LED. The recovery values for the fortified water samples are also shown in Table 4. Satisfactory results were obtained, with recoveries ranging from 84 % to 104 %, thereby demonstrating that the sample matrix does not significantly influence the present method.

4. Conclusions

In this work we showed a palm-sized, affordable device for *in situ* nitrite determination that allows to control and quickly and easily share data to any smartphone or computer without using cables. A RGB sensor was used as a detector and a green LED as a light source, with a self-contained rechargeable battery running up to 7 h on battery. It has been demonstrated through this work that the green LED can be used successfully as a source light to perform an assay, and the platform has been applied to the determination of nitrite in water samples. As a result, a small and inexpensive device was obtained, proving a miniaturized and low-cost method for *in situ* nitrite determination in water, with similar analytical responses when compared to the commercial spectrophotometer.

Additionally, with the use of this device is no need a software or application to convert digital images into RGB values, making the analysis more accessible and faster, improving sample throughput. Finally, the sensor cost ($19) is less expensive than a mid-range smartphone, usually employed for digital image colorimetric analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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