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Infrared Lightbox and iPhone App for Improving Detection Limit of Phosphate Detecting Dip Strips

H. Heidari-Bafroui, B. Ribeiro, A. Charbaji, C. Anagnostopoulos, M. Faghri

Abstract—In this paper, we report the development of a portable and inexpensive infrared lightbox for improving the detection limits of paper-based phosphate devices. Commercial paper-based devices utilize the molybdenum blue protocol to detect phosphate in the environment. Although these devices are easy to use and have a long shelf life, their main deficiency is their low sensitivity based on the qualitative results obtained via a color chart. To improve the results, we constructed a compact infrared lightbox that communicates wirelessly with a smartphone. The system measures the absorbance of radiation for the molybdenum blue reaction in the infrared region of the spectrum. It consists of a lightbox illuminated by four infrared light-emitting diodes, an infrared digital camera, a Raspberry Pi microcontroller, a mini-router, and an iPhone to control the microcontroller. An iPhone application was also developed to analyze images captured by the infrared camera in order to quantify phosphate concentrations. Additionally, the app connects to an online data center to present a highly scalable worldwide system for tracking and analyzing field measurements. In this study, the detection limits for two popular commercial devices were improved by a factor of 4 for the Quantofix devices (from 1.3 ppm using visible light to 300 ppb using infrared illumination) and a factor of 6 for the Indigo units (from 9.2 ppm to 1.4 ppm) with repeatability of less than or equal to 1.2% relative standard deviation (RSD). The system also provides more granular concentration information compared to the discrete color chart used by commercial devices and it can be easily adapted for use in other applications.

Keywords—Infrared lightbox, paper-based device, phosphate detection, smartphone colorimetric analyzer.

I. INTRODUCTION

PHOSPHORUS pollution is a main environmental concern since phosphorus is a critical nutrient in the eutrophication of aquatic ecosystems. Fertilizers and detergents are the foremost artificial sources of phosphorus contamination which impacts many streams, lakes, bays, and coastal waters. Phosphorus, however, is not present in its elemental form because of its high reactivity [1] but rather it is found in the form of phosphates i.e. phosphate rocks [2]. Therefore, it is significant to monitor the phosphate content in water and soil in order to maintain the quality of water in these environments within an acceptable range [3]. Since the 1960s, researchers have been working on finding accurate and easy to use methods for detecting phosphate in aquatic media.

The colorimetric technique is a method in which a color is generated upon its interaction with a specific analyte of interest. This method is generally utilized to determine the concentration of a chemical compound or element in a

solution. The most widespread colorimetric approach currently used for the determination of soluble phosphate in water is the molybdenum blue method introduced by [4]. They suggested a spectrophotometric laboratory method in which phosphate ions first react with an acidic reagent consisting of a mixture of ammonium molybdate and potassium antimony(III) tartrate to generate phosphomolybdate complex. This complex is then consequently reduced by ascorbic acid to form the phospho-antimonyl molybdenum blue (PAMB) compound [5]. The intensity of the blue product formed is directly proportional to the concentration of phosphate present in the solution with higher concentrations of phosphate producing a darker color. Considerable research has been conducted to improve reagent sensitivity, optimize color intensity and formation time, and decrease interferences from other elements for this phosphate detection method [6]-[10].

In the past two decades, paper-based devices have become very attractive for making inexpensive, disposable and convenient analytical devices for the determination of reactive phosphate in the field. Jayawardane et al. [11] proposed a 3-D microfluidic paper-based analytical device (μ PAD) in which two reagents were dried onto filter paper and separated by an interleaving Teflon sheet. A flatbed scanner and desktop-based image processing software, ImageJ, were used to measure the color intensity of the blue complex produced after removing the Teflon sheet and introducing sample into the device. Although μ PADs based on colorimetry are a portable platform for quantitative determination of phosphate in water and wastewater samples, a digital camera or scanner as well as a PC are required to capture and process images. Thus, these kinds of paper-based devices are not fully instrument-free [12]. Furthermore, the storage conditions for reagents and shelf life are challenging issues for these lab-made devices [13], [14]. On the other hand, several highly inexpensive and with over two-year shelf life commercial paper-based test strips are available in the market for detecting phosphate in water. Such devices, however, are only appropriate to be used as initial monitoring purposes [15]. This is the case since only an approximate concentration of phosphate can be measured by commercial test kits due to subjective color comparison by the operator and the discrete color concentrations in the included color chart. Therefore, a digital camera along with a portable colorimetric analyzer is needed to provide more accurate and precise quantitative results.

Recently, use of smartphones for rapid point-of-care testing has been gaining a lot of interest. Additionally, because of the specific features of smartphones such as portability, availability, and ease of use, various mobile-based analytical

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software for specific purposes have been developed on different operating systems (i.e. Android and iOS) [16]-[20]. While some researchers have relied on smartphones for colorimetric analysis and as the illumination source, several reports have proposed a lightbox with light emitting diodes (LED) to provide consistent visible light intensity conditions in order to achieve more reproducible results [21]-[24]. However, to the best of our knowledge there is no report on the use of infrared LEDs in a lightbox to improve the sensitivity of paper-based phosphate analytical devices. Thus, the design and fabrication of an infrared lightbox that enables the capturing of images in the infrared zone while being wirelessly controlled by a smartphone is an attractive system that warrants further development. A combination of lightbox and a smartphone-based colorimetric analyzing application brings about a portable, field-deployable, and low-cost system that enhances the sensitivity of paper-based phosphate devices.

In this work a sophisticated iPhone-based colorimetric analyzer was developed to conveniently and reliably quantify the color measurements of digital photo images by measuring their RGB pixel values. Moreover, a standalone infrared lightbox unit that has an onboard power module, mini wireless router, and a camera without an infrared filter (NoIR), wirelessly controlled by a smartphone was fabricated. The objectives of this research were: (i) To produce an inexpensive, portable infrared lightbox system to create uniform and repeatable lighting environments with an infrared camera to take advantage of the peak absorbance of the molybdenum blue reaction. (ii) To develop an iOS application to measure and analyze the RGB pixel values of pictures. (iii) To present a highly accessible worldwide system for tracking and analyzing field measurements, including location and ambient conditions such as temperature, humidity, wind speed, date, time and time zone, operator ID and other parameters, by developing a data architecture that connects to an online data center. (iv) To combine the aforementioned techniques into a portable reader unit for significant improvements in the measuring capabilities of commercial paper-based phosphate devices, which furthermore act as a substitute for expensive, lab-equipment spectrometers.

II. EXPERIMENTAL

A. Commercial Test Kits

Paper-based phosphate measuring strips are commercially available. While they are convenient and have an acceptable shelf life, they lack sensitivity and only measure phosphate in the parts per million (ppm) range. In this work, two popular commercial devices for measuring phosphate in water, the Indigo phosphate test strips (Indigo Instruments, Ontario, Canada) and the Quantofix phosphate test kit (Macherey-Nagel, Düren, Germany), were tested in the visible and infrared light spectra.

B. Indigo Instruments Phosphate Test Strip

A simple dip strip provided by this supplier produces a blue

colorimetric response upon contact with phosphate ions. The strip is easy to use, and the operating procedure requires the user to only dip the strip into a water sample for one second and then allow color to form in a few minutes. The resulting color on the detection zone can then be compared to that on a provided color chart with 0, 30, 75, 150, and 300 ppm markings.

C. Quantofix Phosphate Test Kit

The Quantofix phosphate dip strip provides an improved detection limit but requires more user involvement and manipulation of acidic reagents and chemicals. The user is required to perform a sequence of steps involving the test strip, reusable vials, provided chemicals, and the water sample before color is produced in the detection zone. The user can then compare the color formed with the provided color patches with markings of 0, 3, 10, 25, 50, and 100 ppm.

D. Solution Preparation

A stock solution of phosphate (100 ppm) was created on the day of testing by dissolving 0.0126 g sodium dihydrogen phosphate (Sigma-Aldrich, MO, USA) in 100 mL of deionized (DI) water. This stock solution was then diluted with DI water to phosphate solutions of concentration 0, 0.1, 0.25, 0.50, 0.75, 1, 2.5, 5, 7.5, 10, 25, 50, and 75 ppm.

E. Infrared Lightbox Unit

The spectrophotometric method is perhaps the standard technique for determining low concentrations of orthophosphate in aquatic solutions based on the molybdenum blue method. He and Honeycutt [25] improved the work of Dick and Tabatabai [26] and they observed that there are two peaks in the absorption spectra of the phosphomolybdate blue complexes. The maximum absorbance peak occurs in the wavelength of around 850 nm which is in the near-infrared range and is 45% higher in value than the other peak occurring at 700 nm in the visible light zone. Recently, some research groups have successfully used the Near-infrared spectroscopy (NIRS) technique to detect phosphorus at lower concentrations in soil or surface water samples [27], [28]. However, their methods are not suitable for on-site measurements or those taken on board ships [29]. Thus, using portable instrumentation to make use of maximum absorption of molybdenum blue in the infrared zone is attractive for improving the analysis capabilities of paper-based devices.

The majority of cameras in the market as well as cellphone cameras are equipped with infrared filters in order to reduce the ambient noise and improve the quality of their pictures. For our lightbox device, a Raspberry Pi 3 Model B+ along with a Pi NoIR Camera v2 and four Everlight LEDs emitting infrared light at 850 nm were used. The NoIR camera is a camera with no infrared filter especially made for Raspberry Pi. A small inexpensive computer board powered by a 2.5 A power supply, the Raspberry Pi can become an operational computer once connected to a monitor, keyboard, and mouse. However, to have a fully portable unit suitable for field measurements, we were able to control the raspberry Pi remotely by cellphone and without use of any peripherals.

This was accomplished via the secure shell method (SSH) which provides access to the Raspberry Pi's command-line interface, and virtual network computing method (VNC) which replicates the graphical desktop, so the cellphone would be used instead of peripheral instruments (i.e. monitor, keyboard, and mouse) for the Raspberry Pi. The cellphone and raspberry Pi can be both connected to the mini wireless router while having the same IP address. The cellphone can then issue commands to the Raspberry Pi to turn on the IR LEDs in the lightbox, activate the camera and to capture one or more images at certain settings. Another command from the cellphone directs the raspberry Pi to transfer the images it stored on its SD card to the cellphone's gallery through the file transfer protocol (FTP). Finally, our colorimetric analyzing app is utilized for instant chemical quantification. Fig. 1 shows a schematic of the lightbox consisting of a raspberry Pi 3, a Pi No IR filter Camera v2, four infrared LEDs, a mini router, and a mini external battery as a chargeable resource for supplying power to the raspberry and the router.

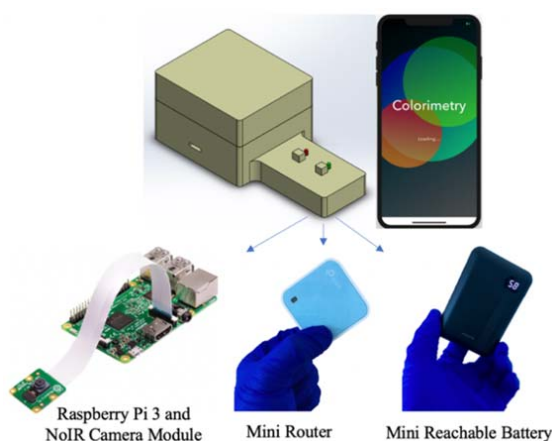


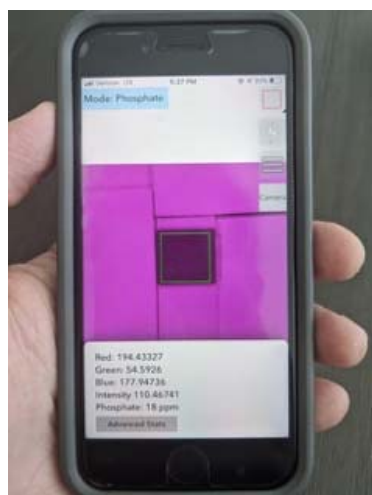
Fig. 1 A schematic of lightbox and its components wirelessly controlled by cellphone

F. Colorimetric Analyzer

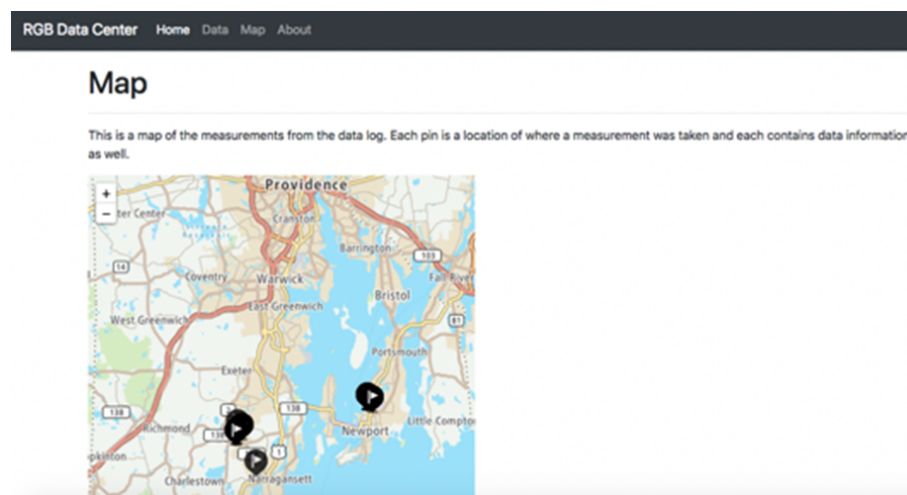
The colorimetric analyzer was built for iOS using Apple's XCode IDE. The application was designed as a simple image processing app capable of analyzing RGB pixel values from photos. The user first picks an image using the app and is then able to analyze the region of interest using an adjustable selection zone. The app then measures the RGB values of all the pixels encompassed by the selection. The average red, green, and blue pixel count along with grayscale is computed for the selection as shown in Fig. 2 (a). These values can then be used to compute phosphate concentrations based on a pre-programmed calibration curve. In addition, pixel histograms are created for even further analysis if necessary. This application was designed with generalization in mind. In other words, the image processing capabilities along with customizable calibration curves can extend the analyzer's use to other colorimetric detection methods and chemicals.

G. Online Data Center

An online data center was constructed in order to interface with measurements taken by the app in the field. Whenever a sample is analyzed in the app, data are stored in the backend with GPS coordinates, humidity percentage, temperature, and phosphate levels. The data can then be uploaded to an online data center that aggregates all the measurements found in a network of devices. This system was built in order to quickly provide insights to phosphate levels of various locations in the field. A heatmap of phosphate levels in a body of water for example, can easily be created using such a system (Fig. 2 (b)). In addition, the storage of scientific data in a centralized database can be used for advanced analytics by both scientists and the public. This procedure encourages collaboration among fellow researchers and accelerates both the collection and interpretation of field data. One can refer to [13] for further details about the app and online data center.



(a)



(b)

Fig. 2 Main view of the colorimetric analyzer app (a) and map page of the online data center

H. Analytical Procedure of the Colorimetric Analyzer

A completely randomized test with three replicates was run for each test kit. All glassware and reusable vials were first washed with a phosphate free detergent. They were then washed with 1M hydrochloric acid and rinsed with DI water three times. The reusable vials of the Quantofix kit were washed with 1M HCl three times, rinsing with DI water in between each wash before every test. The tests were run following the manufacturers' instructions. However, in order to achieve the highest sensitivity of the devices, the effect of reaction time was studied. The images were captured from the detection zone of the devices in the visible light spectrum using a desktop scanner (Canon TS6020) at a resolution of 600 DPI and in the infrared zone by using the lightbox unit. The intensity of the red color for the visible light and the grayscale for the infrared light were then measured to compare the results.

III. RESULTS AND DISCUSSION

A. Effect of the Reaction Time

The suppliers of Indigo and Quantofix test kits have specified the reaction time for their devices. Color produced in the detection zone of Indigo and Quantofix devices should be compared with the provided color scales after 3 minutes and 1 minute, respectively. The reaction time was studied for phosphate concentrations of 50 and 100 ppm for the Quantofix and Indigo test kits, respectively, in which image capturing was performed each minute by the scanner and the lightbox. Fig. 3 (a) shows that the color intensity for the Indigo test kits kept increasing up to 8 minutes and no major changes were observed after that. Thus, reaction time of 8 minutes for this device was selected for further experiments. On the other hand, as shown in Fig. 3 (b), the reaction between the reagent and water rapidly occurred in the Quantofix devices and a gradual decrease of color intensity was observed. Therefore, the images were captured within 1 minute.

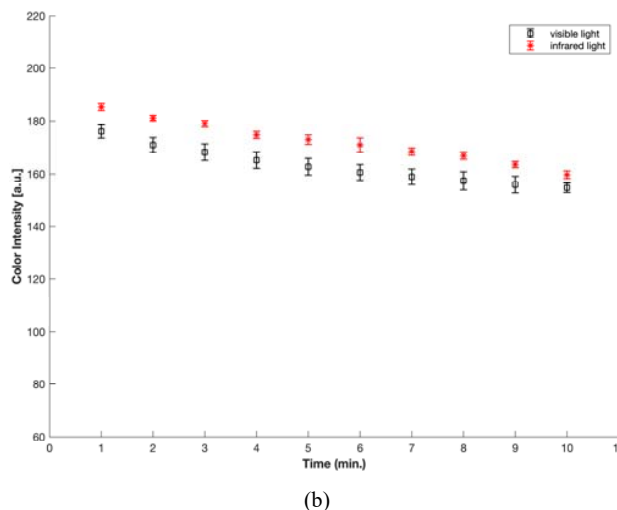
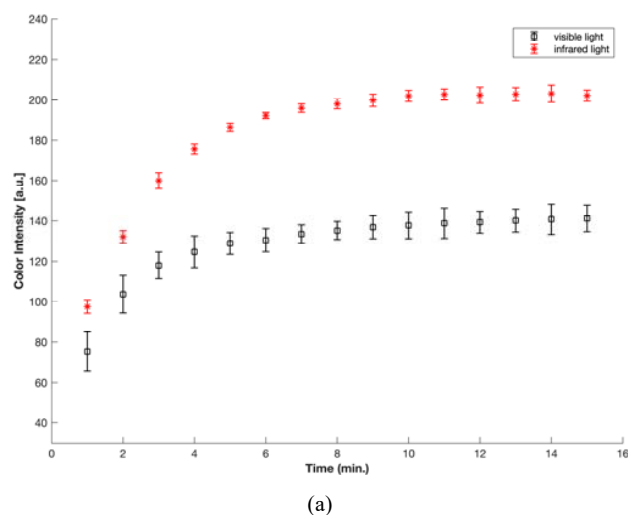


Fig. 3 Effect of reaction time on color intensity in visible and infrared light for Indigo (a) and Quantofix (b) test kits

B. Improvement of Phosphate Test Strips

The advantage of imaging in a remotely controlled infrared lightbox on the analytical performance of two commercial phosphate test kits based on the molybdenum blue method was evaluated. Following suppliers' instructions and the reaction times discussed in 3.1, phosphate working standards (1-100 ppm for Indigo test and 0.1-50 ppm for Quantofix) were measured with three replicates under visible and infrared light conditions. The red color intensity was used to create a calibration curve for the images captured by the scanner since the red intensity shows the largest change over the range of concentrations used. In contrast, due to the lack of visible light in the infrared lightbox, all color channels detect the IR radiation. Thus, the intensity of grayscale was selected to depict the calibration graph for the images captured by the NoIR camera in the lightbox. All values were subtracted by a corresponding blank value and represented in Fig. 4 with a logarithmic x scale along with a linear inset plot for lower phosphate concentrations. As shown in this figure, standard deviations for the infrared data are significantly smaller than those in the visible light. For precision evaluation, 100 ppm phosphate for the Indigo and 50 ppm phosphate for the Quantofix test kit were studied with five replicates. The percent of RSD (%RSD) values for the visible and infrared light were decreased from 3.3% to 1.2% for the Indigo and from 2.1% to 0.51% for the Quantofix test strips, respectively. This proves the reproducibility of light conditions in the box and its superior accuracy for capturing images in the infrared spectrum. Moreover, inset plots in Fig. 4 demonstrate that while the color intensities for visible light in the lower concentrations of phosphate are indistinguishable due to either overlapped intensity values or small slope of increasing values, such differences are quite distinct for those in the infrared light.

MATLAB curve fitting toolbox was used to fit an exponential curve of the form $y = a - b \times \exp(-x/c)$ to the entire range of the data. Table I shows the values of

coefficients for the fitted calibration curves' equations and R^2 values for two test kits in different light conditions. Some researchers [11], [22] split their results into two or three concentration ranges in each of which the intensity of analytes vs. concentrations is linear. Then, they utilize linear equations to find the optimum calibration line for each range and the lower range is used to calculate analytical performance of devices (e.g. limit of detection and quantification). Nevertheless, others may fit a model to the entire range of their analytical data using a nonlinear [30] regression especially an exponential curve [31], [32]. As indicated in Table I, the exponential curves were fitted well to the entire range of data ($R^2 > 0.99$). The obtained calibration equations are being used as a single curve for each condition of the different devices in the developed app as a convenient and reliable analytical reader for the determination of phosphate in the field.

Limit of detection (LOD) and quantification (LOQ) were calculated using formulas: $y_{LOD} = y_{blank} + 3\sigma_{blank}$ and $y_{LOQ} = y_{blank} + 10\sigma_{blank}$ where y_{blank} and σ_{blank} are the mean and standard deviation of the blank sample i.e. 0 ppm [33]. The symbolic math toolbox on MATLAB was used to find the concentrations that gave the y_{LOD} and y_{LOQ} values calculated by the fitting functions used. Table II summarizes the LOD and LOQ calculated for two commercial test strips in the visible and infrared light. An LOD and LOQ of 9.2 and 39.2 ppm phosphate respectively, were obtained using the colorimetric method with visible light for the Indigo test strips. The LOD and LOQ were reduced quite significantly to 1.4 and 7.9 ppm phosphate respectively, in the infrared light, improving the sensitivity of the device by approximately a factor of 5. Similarly, Quantofix devices had an LOD and LOQ of 1.3 and 4.5 ppm phosphate in the visible light. However, these values were improved by a factor of 4 in the infrared light and phosphate concentrations were detected and quantified at 0.3 and 1.2 ppm respectively. This improvement is not limited to the commercial devices and can be applied to any paper-based devices that use the molybdenum method to detect phosphate in water or soil.

Comparing with relevant literature, Jayawardane et al. [11] reported a paper-based device with a working range of 0.6-30 ppm phosphate and can detect phosphate in the parts per billion range. However, their device is not a fully instrument-free portable device and shelf life is a main concern since it ranged from a couple of days in room temperature to around 112 days at temperatures below -20°C . These issues are not of concern for commercial devices since their shelf life is at least

two years. Using the affordable Quantofix test kits and the portable infrared lightbox, one can detect phosphate in parts per billion range in the field.

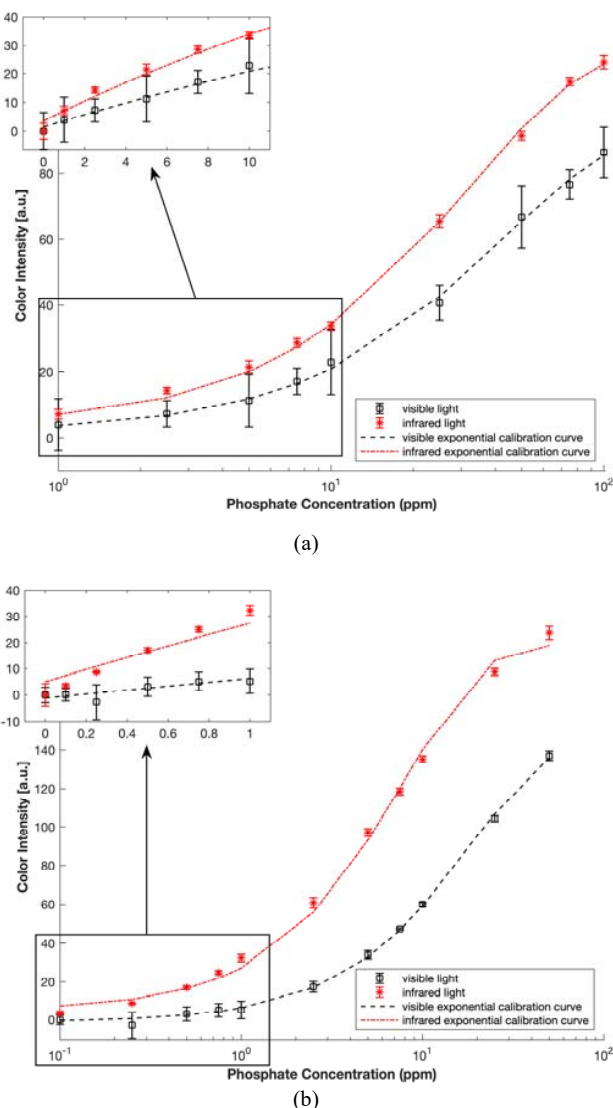


Fig. 4 Calibration curves for phosphate response in the visible and infrared light conditions for the Indigo (a) and Quantofix (b) test strips

TABLE I
COMPARISON OF THE COEFFICIENTS (WITH 95% CONFIDENCE BOUNDS) AND THE DETERMINATION COEFFICIENTS (R^2) FOR THE EXPONENTIAL CURVE ($Y = A - B \cdot \exp(-X/C)$) FIT TO THE VISIBLE AND INFRARED DATA

Test kit	Visible light				Infrared light			
	a	b	c	R^2	a	b	c	R^2
Indigo	94.5 (± 6.7)	93 (± 6.2)	42.94 (± 7.60)	0.998	118 (± 5.8)	114.4 (± 5.4)	32.22 (± 4.78)	0.998
Quantofix	146.7 (± 6.1)	147.9 (± 5.8)	19.12 (± 1.82)	0.999	194.1 (± 9.2)	188.9 (± 9.9)	7.98 (± 1.11)	0.997

TABLE II
 COMPARISON OF LOD AND LOQ IN THE VISIBLE AND INFRARED LIGHT CONDITIONS

Test kit	LOD (ppm)			LOQ (ppm)			Testing range (ppm)
	Visible light	Infrared light	Progress factor	Visible light	Infrared light	Progress factor	
Indigo	9.200	1.429	6.4	39.154	7.917	4.9	1-100
Quantofix	1.352	0.337	4	4.479	1.182	3.8	0.1-50

IV. CONCLUSIONS

The portable and low-cost infrared lightbox developed in this paper along with the iPhone-based analyzer app are of great significance since they demonstrate that complex sensors and expensive spectrometers are not required to take advantage of the infrared spectra. The absorption peak of the molybdenum blue reaction in the infrared region was utilized by a remotely controlled lightbox. This leads to a significant improvement in the LOD and LOQ of two common commercial devices by a factor of up to 6. LOD decreased from 9.2 to 1.4 ppm for the Indigo strips and from 1.35 to 0.34 ppm for the Quantofix phosphate test strips. Moreover, maintaining constant illumination on samples while capturing images by the lightbox provided accurate and repeatable results with RSD values less than 1.2%. This approach is not exclusive to the detection of phosphate since the lightbox and colorimetric analyzer can be further developed in order to account for other colorimetric reactions such as nitrate and nitrite. Additionally, the infrared lightbox can be used to improve sensitivity of other commercial as well as lab-made paper-based devices.

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