



A Low-Cost Video-Based Reflectometer for Selective Detection of Cu²⁺ Using Paper-Based Colorimetric Sensors

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This paper presents a low-cost reflectometer for selective detection of Cu²⁺. The reflectometer is based on an emission light source using five light-emitting diodes at different wavelengths and a detector using a webcam. The samples were prepared using a filter paper-based colorimetric sensor with ascorbic acid-based quinoxaline derivative. Video analysis using gray color intensity was used to perform both copper ion screening (Cu²⁺, Pb²⁺, Cr³⁺, Ni²⁺, Fe²⁺, Sn²⁺, Mg²⁺, Cd²⁺, Ag⁺ and Co²⁺) and quantification of Cu²⁺ in spiked samples. In addition, a multivariate validation was performed. The system proved to be selective to Cu²⁺ among the screened ions, and its quantification was performed using partial least square regressions. Good linearity (R² = 0.979, coefficient of determination), low root-mean-square error of prediction (RMSEP = 5.20 × 10⁻³ mol L⁻¹) and high recovery (93.39–128.64%) were achieved. This method has potential to be employed for rapid and selective determination of Cu²⁺ in water using low-cost instrumentation.

Keywords: reflectometer, webcam, paper-based colorimetric sensor, low-cost instrumentation, Cu²⁺

Introduction

The design, fabrication and feasibility of portable handheld analytical instruments for detection and quantification of different classes of compounds are reported in the literature.¹ These instruments are useful and advantageous, especially due to the low cost, simplicity of operation, convenience of fast *in situ* analysis and appropriate sensitivity and specificity to produce near real-time information used to solve specific problems which require rapid feedback of information.² Examples of portable instruments based on ion trap mass,³ Raman,⁴ near infrared⁵ and mid-infrared⁶ spectroscopies, capillary electrophoresis⁷⁻¹¹ and X-ray fluorescence¹² represent the tendency of instrument miniaturization for sensing applications. In case of miniature ion trap mass spectrometry, which represents a high breakthrough in portable analytical instrumentation, two approaches can be used to build this kind of instrument: the bottom-up approach in which the miniature instrument is assembled with the components built on a specific scale of interest; and the top-down approach, in which the component sizes of a macroscale instrument are reduced in an interactive way to maintain their performance. Bottom

up instruments are usually concentrated on quadrupole mass filter analyzers, microelectromechanical systems (MEMS) methods, or hybrid technology such as those utilized in the Chem-Cube/ChemPack instrument; whilst top-down instruments usually have simplified cylindrical or rectilinear ion trap geometry having reduced components, such as a small radio frequency (RF) supply and vacuum system, ensuring low size, weight, and power consumption. Examples of this type of instrument was developed by Ouyang *et al.*³

Undoubtedly, light-emitting diodes (LED) represent the most commonly applicable light source in handheld instrumentation.¹³ LED photometers¹⁴ possess main characteristics of: simplicity, low cost, low power consumption and portability, which make them useful for *on site* measurements. Multi-LED photometers, for instance, may be sufficient for analysis or discriminatory simultaneous determinations based on multivariate analysis.¹⁵ In the last decade, webcams,¹⁶ digital cameras¹⁷ and mobile phone cameras¹⁸ have been reported as an alternative detector unit for chemical/biochemical analysis systems for portable instruments. These image detectors are based on the RGB (red-green-blue) color system and present the color of emergent radiation. The images are retrieved in the three individual R, G and B components,

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which may be addressed solely or even combined between themselves in attempt to promote a broader spectrum of applications.

Although reflectance has proven to be an interesting tool for many applications, there are only few reports associated to the detection of ionic analytes based on this technique.¹⁹⁻²¹ In fact, UV-Vis and fluorescence spectroscopy are the main techniques applied in studies involving chemosensors for ionic analytes.^{22,23} In attempt to demonstrate the feasibility of reflectometer devices presented in this work for detection and colorimetric quantification of cationic analytes, we performed a study using a disposable paper-based sensor containing the compound *N*-(2-aminophenyl)-3-((1*S*,2*S*)-1,2,3-trihydroxypropyl)quinoxaline-2-carboxamide (AAQX) adsorbed onto it. An explanation of the electronics of a homemade handheld portable reflectometer is presented, being used an inexpensive webcam detector for monitoring the chemical reaction that produces color change.

Paper devices including paper strip tests, paper-based assays and paper-based microfluidics present some advantages such as being available everywhere, low-cost, thinness, lightweight, easy to stack, store and transport.^{24,25} For instance, paper-based devices have been successfully applied in biological testing,²⁶ environmental analysis,²⁷ drug detection^{28,29} and heavy metal detection.³⁰ As the paper is usually white (because it scatters light), colorimetric testing is of great potential because it provides a strong contrast with a colored substrate.³¹ For colorimetric sensing, analyte flow is directed along the paper matrix by capillary action, and the analyte then reacts with a label or an organic dye in the test zone.³² The choice for this quinoxaline derivative was based on previous work from our research group in which naked-eye and UV-Vis analyses showed AAQX selective for the Cu²⁺ detection in methanolic solution against several other cationic species.³³ In this context, as a subsequent step, we aimed to investigate the possibility of selective response to Cu²⁺ when this compound is coated on paper. Naked-eye and reflectance analyses were able to detect the referred analyte, with the latter being applied for quantification purpose. Finally, this paper aims at validating the video metric analysis method based on RGB frames to estimate the linearity, precision, sensitivity, bias and recovery.

Experimental

Colorimetric LED reflectometer

Microcontroller

The reflectometer was based in an Arduino UNO plate with an ATmega328 microcontroller chip. Additionally,

the Arduino plate has a working tension of 5 V, input tension between 7 and 12 V, 14 digital I/O pins, 6 digital analogical pins, DC current *per* I/O pin of 40 mA, flash memory of 32 kB, SRAM memory (or data memory) of 2 kB, EEPROM memory (or flash memory) of 1 kB, and a clock speed of 16 MHz.

Detection

The detection system was composed of a Microsoft® LifeCam VX-800 webcam with resolution of 640 × 480 pixels (CMOS VGA sensor technology, 59° diagonal field of view).

LED array

The samples were irradiated with five standard LEDs of 3.0 V and 20-25 mA with yellow (570-590 nm), green (500-570 nm), red (610-780 nm), white (450-780 nm) and blue (450-500 nm) color emissions. Each LED was individually actioned during a period of 3 s according to the sequence previously mentioned.

Reflectometer overview

Overall, the reflectometer was simply based on three different systems: (i) LED radiation source, (ii) sample holder and (iii) a detector based in a webcam. A resistor of 6.8 kΩ was used to control the current of the radiation source actioned by the Arduino plate. The Arduino worked as a control system of the radiation source, where the LEDs activation sequence and elapsed time were controlled by this device. The sample holder was based on a cropped plastic container to hold the paper strip in vertical position and to expose its main part to the radiation source. It holds the paper-based sensor with size of 2.0 × 4.0 cm (see Supplementary Information (SI) section). The webcam detector was directly connected to a personal computer through a serial USB interface where video files were recorded using MyCam version 1.1 software (e2eSoft) after the LEDs were actioned. The recording time of the videos was constant and equal to 36 s. The reflectometer overview is shown in Figure 1 and the cost to build the reflectometer device is shown in Table 1.

Computational analysis

The video of each sample was processed using MATLAB® R2012b software (MathWorks, USA) with PLS Toolbox 7.0.3 (Eigenvector Research, Inc., USA). The videos were imported into MATLAB environment and its gray color intensity was extracted during the time period of 36 s (161 frames). The gray color intensity (I_{gray}) is based on the RGB (red-green-blue) response for each frame according to equation 1:³⁴

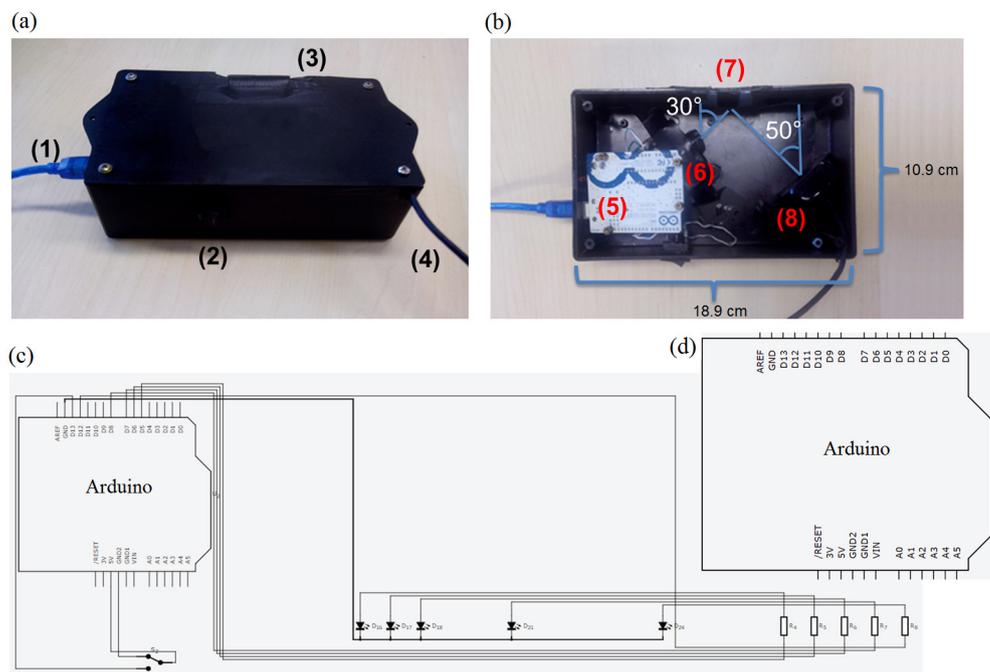


Figure 1. (a) Outside view of reflectometer device: (1) power USB, (2) turn on/off switch, (3) sample holder lid and (4) video USB; (b) inside view of reflectometer device: (5) Arduino UNO plate (which is originally screwed into the reflectometer lid), (6) LED array, (7) sample holder, and (8) webcam; (c) reflectometer electronic circuit; and (d) Arduino plate from zoomed circuit.

Table 1. Cost of the components used to build the reflectometer

Component	Price / US \$
Plastic container	2.00
Arduino Uno	24.95
LEDs ^a	0.12
Resistors of 6.8 kΩ ^a	0.19
Switch button	0.45
Webcam	21.74
Total	49.45

^aPrice for five units.

$$I_{\text{gray}} = 0.30I_{\text{R}} + 0.59I_{\text{G}} + 0.11I_{\text{B}} \quad (1)$$

where I_{R} , I_{G} and I_{B} are the red, green and blue intensities, respectively.

This gray color intensity is related to the reflectance of the paper-based sensor, according to equation 2, where the Kubelka-Munk function is represented by:³⁵

$$f(R) = \frac{(1-R)^2}{2R} = \frac{k}{s} \quad (2)$$

where R is the reflectance at some path, k is the particle-size-dependent absorption coefficient, and s is the scattering coefficient.

The reflectance as color intensity for each frame during the analysis time was arranged into a row-vector \mathbf{x}_i , representing the analytical signal for a sample i . These

signals were concatenated into a \mathbf{X} $\{m \times n\}$ matrix and preprocessed by auto-scaling normalization prior to further analysis using both screening and quantification approaches.

Copper screening was performed using principal component analysis (PCA). PCA scores were used as the signal indicator of the related metal. The quantitative analysis was performed using partial least square (PLS) regressions with the preprocessed \mathbf{X} matrix. For this, the data were divided into calibration (70%) and prediction (30%) sets by using Kennard-Stone sample selection algorithm.³⁶ The model was built using cross-validation venetian blinds. The image method was evaluated and validated according to the following figures of merit: root-mean-square error of cross validation (RMSECV) and prediction (RMSEP), linearity, precision, sensitivity, bias and recovery.

Paper-based colorimetric sensor

Synthesis of organic colorimetric sensor

The compound AAQX was synthesized according to literature and their spectroscopic data were coherent to the proposed structure.^{33,37}

Paper-based sensor preparation

In order to develop a rapid, low cost and simple analytical procedure, strips of grade 1 Whatman® qualitative cellulose

filter paper were used as paper-based sensors. These strips had dimensions of 2.0×4.0 cm with $180 \mu\text{m}$ thickness. The paper-based sensor was prepared by soaking the filter paper strips in the solution of AAQX ($2.00 \times 10^{-3} \text{ mol L}^{-1}$) for 5 s. Then, the strips were immersed separately in solutions containing pure water and $2 \times 10^{-2} \text{ mol L}^{-1}$ of different cations (Pb^{2+} , Cr^{3+} , Ni^{2+} , Fe^{2+} , Sn^{2+} , Mg^{2+} , Cd^{2+} , Ag^{+} , Co^{2+} and Cu^{2+}) for 5 s. Quantification of Cu^{2+} in solution was performed by soaking strips of paper-based sensors in solutions of the referred cations in concentrations ranging from 2.00×10^{-5} to $6.00 \times 10^{-2} \text{ mol L}^{-1}$. The visual strip test is based on a change of color when a chemical reaction between the analyte and the utilized reagent takes place. This color change is proportional to the analyte concentration.

Results and Discussion

Synthesis and copper ion screening

A synthetic quinoxaline-based compound (AAQX) was utilized as a colorimetric sensor adsorbed onto the filter paper. This compound was easily synthesized through a one pot procedure, starting from initial oxidation of L-ascorbic acid into dehydroascorbic acid followed by its reaction with *o*-phenylenediamine.^{33,37}

Our previous work showed that AAQX selectively interacts with Cu^{2+} in methanolic solution, and this reaction was able to be followed through naked-eye and UV-Vis analyses.³³ On the other hand, AAQX is a polyfunctionalized molecule containing alcohol, amine, amide and quinoxaline moieties, which are able to interact with available hydroxyl groups of the cellulose fibers contained in paper via hydrogen bonds, and this fact opened a possibility for immobilizing AAQX on a paper support in attempt to develop a disposable sensor. For decades, the literature has been presenting examples in which hydrogen bonds are the main interaction in the adsorption of polyols, phenols and amines, among other chemical species to cellulose chains,³⁸⁻⁴² strongly suggesting that this type of interaction is involved in the adsorption mechanism of AAQX to the paper strips. One important feature related to AAQX is its low solubility in water, which ensures that the chemosensor remains adsorbed to the paper strip after immersion in aqueous solution.

When the paper strip containing adsorbed AAQX was dipped into 10 mL of an aqueous solution of $2.00 \times 10^{-3} \text{ mol L}^{-1} \text{ Cu}^{2+}$, the color of the paper immediately changed from colorless to yellow. However, no significant changes in color were verified for the other metals in the same conditions (see Figure S2 in the SI section).

Reflectometer analysis

In diffuse reflectance measurements, the reflectometer reads a signal of the diffused reflected radiation. For the homemade reflectance device used in this study, the reflected radiation signal was represented by the gray color intensity acquired from a sample being irradiated with different wavelengths. The video-based reflectance signal acquired by the reflectometer was obtained from the gray color intensity of each frame in the video. The signal of paper-based colorimetric sensor in the presence of Cu^{2+} and other metal ions is very similar to each other (Figure 2a). The main observable difference is in the profile of the most intense peaks, which are slightly sharper in the presence of Cu^{2+} ions (see Figure S5 in the SI section). In addition, although the signals recorded are similar to the blank signal (Figure 2b), the signal is slightly more intense when AAQX is in the presence of metal.

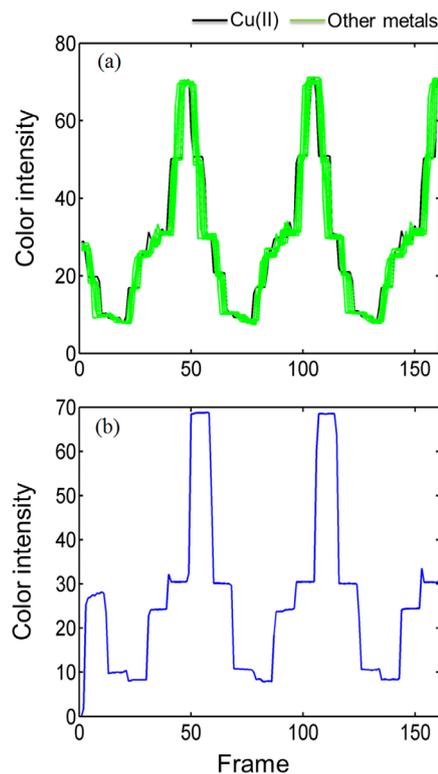


Figure 2. Video-based reflectance signal acquired for different metal ions (green: Pb^{2+} , Cr^{3+} , Ni^{2+} , Fe^{2+} , Sn^{2+} , Mg^{2+} , Cd^{2+} , Ag^{+} and Co^{2+} ; black: Cu^{2+}), and (b) video-based reflectance signal for blank sample having the paper-based sensor (AAQX) without any metal.

The noise acquired with the LEDs off (only the webcam recording) was very small, with a magnitude to the order of 10^{-3} (see SI section), being negligible when compared to the analyte signal which had a magnitude to the order of 10^1 . Therefore, the reflectometer device was appropriately sealed, so that external radiation sources do not significantly

affect the recorded signal. The stability of the paper-based colorimetric sensor according to its exposure time to the environment and when it was dipped in deionized water is shown in Figure 3.

As can be seen in Figure 3a, the color signals of the paper-based colorimetric sensor have small intensity variations when measured on different days, showing an

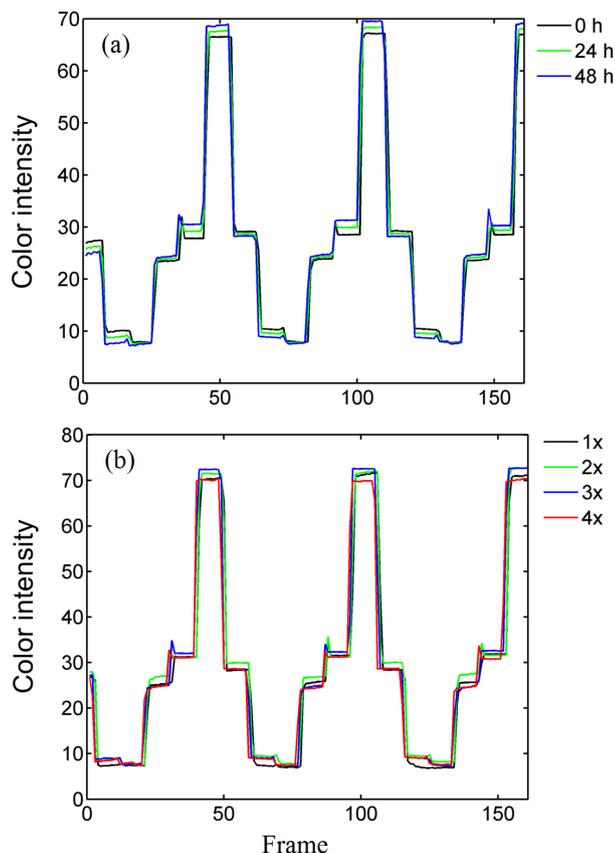


Figure 3. (a) Reflectance color signal for the same sample acquired at different time periods: 0, 24 and 48 h, and (b) reflectometer gray color signal for the same sample being 1 \times , 2 \times , 3 \times and 4 \times dipped in water.

average standard deviation of 1.32 intensity units in the whole color signal (coefficient of variation of 0.049) with 48 h of exposure time. However, the color signal tends to increase as times passes, therefore, the best procedure was to measure the sample as soon as the paper-based sensor is ready for analysis. Regarding dipping the paper-based sensor in deionized water, the color signals have some small deviations (Figure 3b). The average standard deviation in the whole color signal was equal to 2.10 intensity units (coefficient of variation of 0.072) with four immersions in water. Therefore, the sensor and the analyte seem to be well fixed to the paper and no content loss was systematically observed.

The copper screening result is shown in Figure 4. PCA scores for the samples of Cu^{2+} ions ($2.00 \times 10^{-2} \text{ mol L}^{-1}$) are differentiated from the others, presenting higher values of scores on PC 2 (25.22% of explained variance). As can be seen in Figure 4b, the score values on PC 2 were only above 5 in presence of Cu^{2+} , while most of the others were negative. In fact, these results are coherent to the response of AAQX toward different cationic species in solutions verified using UV-Vis analysis.³³

Quantification of Cu^{2+}

Initially, a univariate calibration attempt was made to build a calibration curve for determining Cu^{2+} concentration according to its reflectance signal. However, using the best univariate curve ($R^2 = 0.892$, coefficient of determination) (as determined according to a homemade algorithm created to select the best variable from the **X** matrix univariately related to the concentration), the prediction error was considered unsatisfactory ($\text{RMSEP} = 1.83 \times 10^{-2} \text{ mol L}^{-1}$). Therefore, we preferred to use a multivariate calibration approach using partial least square (PLS) regressions

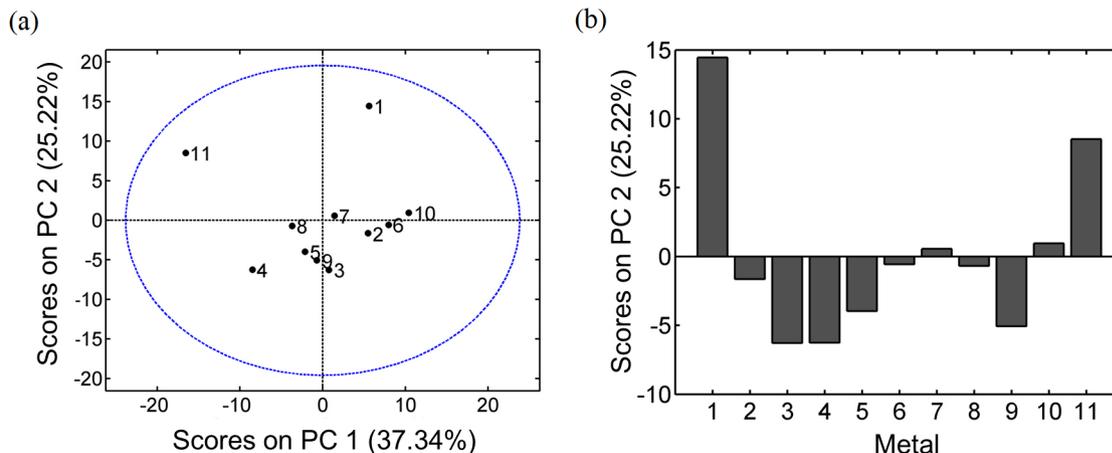


Figure 4. (a) PCA scores within a 95% confidence level ellipse (blue circle) for copper ion screening based on its reflectometer signal and (b) bar chart with the scores on PC 2 for: (1) Cu^{2+} , (2) Pb^{2+} , (3) Cr^{3+} , (4) Ni^{2+} , (5) Fe^{2+} , (6) Sn^{2+} , (7) Mg^{2+} , (8) Cd^{2+} , (9) Ag^+ , (10) Co^{2+} and (11) all metals mixed.

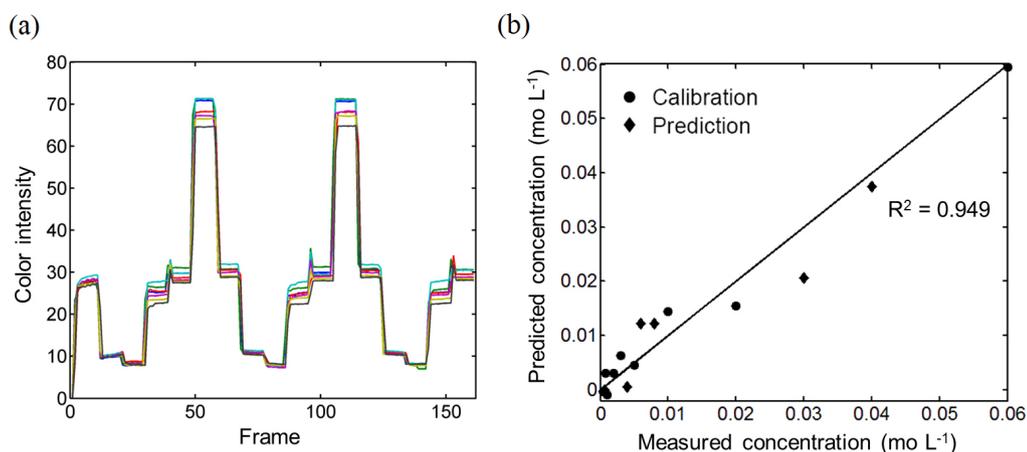


Figure 5. (a) Reflectance color signals for Cu^{2+} solutions in different concentrations (2.00×10^{-5} - 6.00×10^{-2} mol L^{-1}), and (b) experimentally measured concentration of Cu^{2+} vs. predicted concentration of Cu^{2+} by PLS model for calibration (\bullet , $n = 13$) and prediction (\blacklozenge , $n = 6$) sets.

with the whole signal since the prediction error was 10 times smaller. Therefore, the quantification of Cu^{2+} in aqueous solution (2.00×10^{-5} - 6.00×10^{-2} mol L^{-1}) was performed using PLS regression (4 latent variables, 97.98% of explained variance). The regression model was performed within the linear concentration range found for the Cu^{2+} samples: 2.00×10^{-5} - 6.00×10^{-2} mol L^{-1} . Below this concentration range, the signal is not bright enough to differentiate it from the blank sample; and above this concentration range, the signal saturates. The signals for the Cu^{2+} samples within the linear concentration range are shown in Figure 5. In addition, a paired t -test was performed between the predicted and the measured concentrations and no statistical difference was observed at a confidence level of 95%. Table 2 summarizes the figures of merit (FOM) used to evaluate the quality of the PLS model.

As can be seen in Table 2, satisfactory results were observed for linear response, recovery, precision, limits of detection and quantification (LOD and LOQ, respectively) and analytical sensitivity for quantification of Cu^{2+} using the PLS model. The linear range in terms of Cu^{2+} concentration was higher than other values reported in the literature,⁴³⁻⁴⁵ in which the proposed method was able to measure Cu^{2+} concentration up to 3.81 g L^{-1} ; the recovery was very close to previously reported values in the literature (92.60-119.01%).⁴³ The precision in terms of relative standard deviation (RSD) was a little higher than previously reported values (7.1-9.5%),⁴³⁻⁴⁵ which may be caused by a lower signal-to-noise ratio of the webcam in comparison to spectrometric measurements. Also, the limit of detection for Cu^{2+} was higher than other values reported in the literature (usually in the range of mg L^{-1} to $\mu\text{g L}^{-1}$).⁴³⁻⁴⁶ These lower values of limit of detection are achieved using more complex paper-based analytical devices, such as using metal nanoparticles or more complex manufacturing

Table 2. Quality parameters determined for PLS model

Calibration parameter	
Number of latent variables	4
Explained variance / %	97.98
Linear range / (mol L^{-1})	2.00×10^{-5} - 6.00×10^{-2}
RMSECV / (mol L^{-1})	2.30×10^{-3}
Bias at calibration / (mol L^{-1})	0.00
Prediction parameter	
RMSEP / (mol L^{-1})	5.20×10^{-3}
Bias at prediction / (mol L^{-1})	9.00×10^{-4}
Linearity	
Slope	0.979
Intercept	1.60×10^{-4}
R	0.989
R^2	0.979
Other FOM	
Precision ^a / %	11.45
Recovery / %	93.39-128.64
LOD / (mol L^{-1})	2.12×10^{-4}
LOQ / (mol L^{-1})	7.11×10^{-4}
Analytical sensitivity (γ) / (L mol ⁻¹)	4.13×10^3
γ^{-1} / (mol L^{-1})	2.42×10^{-4}

RMSECV: root-mean-square error of cross validation; RMSEP: root-mean-square error of prediction; R: correlation coefficient; R^2 : coefficient of determination; FOM: figures of merit; LOD: limit of detection; LOQ: limit of quantification. ^aPrecision calculated as relative standard deviation for six replicates.

techniques.⁴³⁻⁴⁶ The type of detector also improves the limit of detection, as demonstrated using UV-Vis spectroscopy.⁴³ On the other hand, the proposed method in this paper is very low-cost, simple and portable, presenting reliability and feasibility to analyze Cu^{2+} in spiked water samples. In addition, the video-based reflectometer can be used to analyze other metal ions by changing the type of paper sensor and the calibration curve utilized.

Conclusions

A simple homemade reflectometer based on an array of LEDs was investigated in determining Cu²⁺ in aqueous solution. In this proposal, a paper-based colorimetric sensor containing an organic chromophore (AAQX) was applied for quantitative colorimetric reflectance analysis. The obtained results clearly show that the method is relatively easy to be performed and presents good analytical results. The referred reflectometer was constructed from low price and commercially available components, such as LED, an Arduino microcontroller and a webcam. Finally, our method was able to demonstrate that this light visible-based reflectometer is quite feasible for application in the selective detection of Cu²⁺, even with water coated onto the paper.

Supplementary Information

Supplementary information is available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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