Sensitive Colorimetric Analysis Of Nitrite Ions Using A Smartphone

M. Lutfi Firdaus, Hadi Apriyoanda, Juwita Megarani, Thimarana N. Pratiwi, Hermansyah Amir, Agus Sundaryono

Abstract: Here, we report the development of digital image-based colorimetry combined with a smartphone application to analyze nitrite ions in aqueous solution. Initially, nitrite was diazo-coupled with p‑aminobenzoic acid and phloroglucinol to produce a bright-yellow color. The change of yellow color is linearly proportional with nitrite concentration change. These color changes were then recorded using a smartphone camera as raw data to perform a digital image-based colorimetry. Furthermore, a smartphone application, namely 'QAnalytics' was built as a detector replacing any other analytical instruments to make the proposed method portable. By using the proposed method, we are able to achieve a detection limit of 3.2 ppb which is better than the permissible nitrite concentration in drinking water of 0.2 ppm that was set by World Health Organization (WHO). The method was tested to real samples that give satisfactory results on accuracy (2.5%) and precision (4.9%). From these results, the developed method proved to be sensitive, low-cost, simple, and portable for nitrite ions quantification and thus can be applied in remote areas with limited resources.

Index Terms: Colorimetry, digital image, nitrite, smartphone application, spectrophotometry.

1 INTRODUCTION

NITRITE ions (NO_2^-) are naturally occurring in the environment as parts of the nitrogen cycle. By volume, dry air contains 78.1% nitrogen (N_2) that is the most abundant compounds over the entire atmosphere [1], [2]. Bacteria, such as azotobacter and rhizobium, convert atmospheric nitrogen into ammonia (NH_3) through the so-called 'nitrogen fixation' process [1]. In addition, human fixed atmospheric nitrogen through industrial fixation by manufacturing ammonia in high pressure and temperature reactor for various industrial purposes such as agriculture fertilizer [3]. The next nitrogen cycle stage, nitrification, occurs in two steps as follow; (1) the conversion of ammonia to nitrite by nitrosomonas and nitrococcus bacteria present in the soil, and (2) nitrite conversion to nitrate (NO_3^-) by nitrobacter bacterium [1]. In the present oxic atmospheric condition, nitrite is not stable and will soon be converted to nitrate through the oxidation process. The last nitrogen cycle stage, called denitrification, is the reverse of nitrification that converts nitrate back to nitrogen and other gaseous compounds like nitrogen dioxide (NO_2) by anaerobic denitrifying bacteria [1].

Among nitrogen compounds, nitrite is the most toxic to human because it interferes the function of hemoglobin in oxygen uptake that eventually induces human's tissue damage as a result of oxygen deficiency [4], [5]. Nitrate in the human body is changed to nitrite through salivary glands [4]. Therefore, exposure of both nitrate and nitrite could be fatal to a human. The US Environmental Protection Agency (EPA) has set a maximum contaminant level of nitrite in the water at 1 ppm that is more stringent than nitrate (i.e. 10 ppm) [6].

Furthermore, the World Health Organization (WHO) set the maximum nitrite values in drinking water at 0.2 ppm for long-term exposure [7]. Based on this background, it is crucial to develop a sensitive analytical method to detect and monitor nitrite concentration in drinking water and environments.

Many attempts have been made to develop an analytical method toward nitrite quantification, such as spectrometry [8], [9], chromatography [10], fluorescence [11], [12], flow injection analysis [13]. However, most of these methods are high cost, required a lot of chemicals, time-consuming and laborious procedure. Colorimetry is the simplest method to detect chemical compounds that relied on the color change of interest analyte. Since nitrite has no color, it must be reacted first with a chromogenic agent to make it colorful. There are several agents that have been used to colorize nitrite like sulfanilic acid [9], tetrahydroquinoline [14], sulfanilamide (Griess reagent) [15],[16],[17] and tetrazine [18]. Here, we propose a low-cost, simple, and sensitive method to detect nitrite ions in aqueous solution using p‑aminobenzoic acid as a chromogenic agent coupled with colorimeter attached in a smartphone application.

2 MATERIALS AND METHODS

All chemicals used in this study were of analytical grade or the highest purity available. Phloroglucinol (1, 3, 5‑trihydroxybenzene), p‑aminobenzoic acid (PABA), and salts of NO_2^-, NO_3^-, CO_3^-, HCO_3^-, Cl^-, SO_4^{-2}, S^{-2}, SCN^-, $H_2PO_4^-$, SiO_3^{-2}, $B_4O_7^{-2}$ and $C_2O_4^{-2}$ were purchased from Merck Ltd. (Darmstadt, Germany). Working standards in the range of ppb to ppm were prepared by dilution on a weight basis. All glassware was cleaned with detergent (5%), 4M HCl and deionized water before use.

Samples were put in cuvettes and photographed using a smartphone Vivo Y65 (Vivo Electronics, Guangzhou) in a custom-made mini photo studio. Post-processing of the photograph as the digital image was initially done using a computer with various software such as Photoshop (Adobe Inc., San Jose, CA, USA) for cropping and adjustment and MATLAB (MathWorks) for Red – Green – Blue (RGB) color value extraction. Android Studio (Google Inc., Mountain View, CA, USA) was used to build a smartphone application.

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A more detailed method has been published elsewhere [19].

A linear plot of digital image was initially obtained using a computer that converted to an application code attached in a smartphone. The RGB color values were transformed to log-scale to get a linear form, following derivation of the Lambert-Beer equation below [20]:

$$I_R = \log \frac{R_0}{R_s}$$

$$I_G = \log \frac{G_0}{G_s}$$

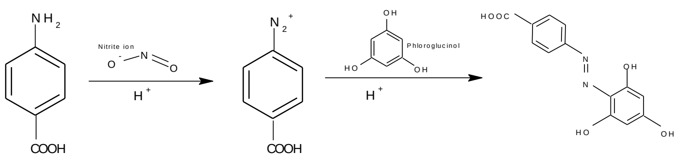
$$I_B = \log \frac{B_0}{B_s}$$

The notation of IR, IG, and IB indicates the color intensity equal to color absorbance for red, green and blue, respectively. R0, G0, B0 and Rs, Gs, Bs are the RGB color values of the blank and the sample, respectively. It should be pointed out here that the term 'intensity' refers to the value of RGB color of the digital image which is different from that used in spectrophotometry. The log conversion of RGB color values is proportional to the nitrite ions concentration, thus it can be used for nitrite ions quantification in the samples. As a reference method, absorption spectra were measured using a Visible Spectrophotometer (B-One, Messgerate).

3 Results and Discussion

3.1 Color formation and smartphone application

We chose the approach of diazo-coupling reaction using p-aminobenzoic acid (PABA) and phloroglucinol to colorize nitrite ions that are recently reported by Ibrahim et al. [8]. The colorimetric reaction of reagents with nitrite ions was conducted at pH 2 that produced a bright-yellow azo dye with maximum absorbance at 430 nm wavelength. This approach was selective only to nitrite ions with the chemical reaction as follow:



PABA Diazonium ion Azo dye

In this research, we aim to provide an alternative instrument for nitrite detection using a ubiquitous smartphone. The smartphone application was home-made using a freeware Android Studio. Example of code and application appearance is shown in Figure 1. The instruction for nitrite determination is embedded in the application. First, select the "Detection menu" found in the main window and then select the "Camera menu" to take a picture of 0.1 mL sample that has been added with 1 mL PABA 0.5%, 0.5 mL HCl 8M and 1 mL phloroglucinol 0.6%. After pressing the "Gallery menu" to choose the previously shot digital image of the sample and adjust its cropping area, pressing the "Detect button" will show us the Blue channel value, Absorbance value (Blue Intensity) and Nitrite concentration.

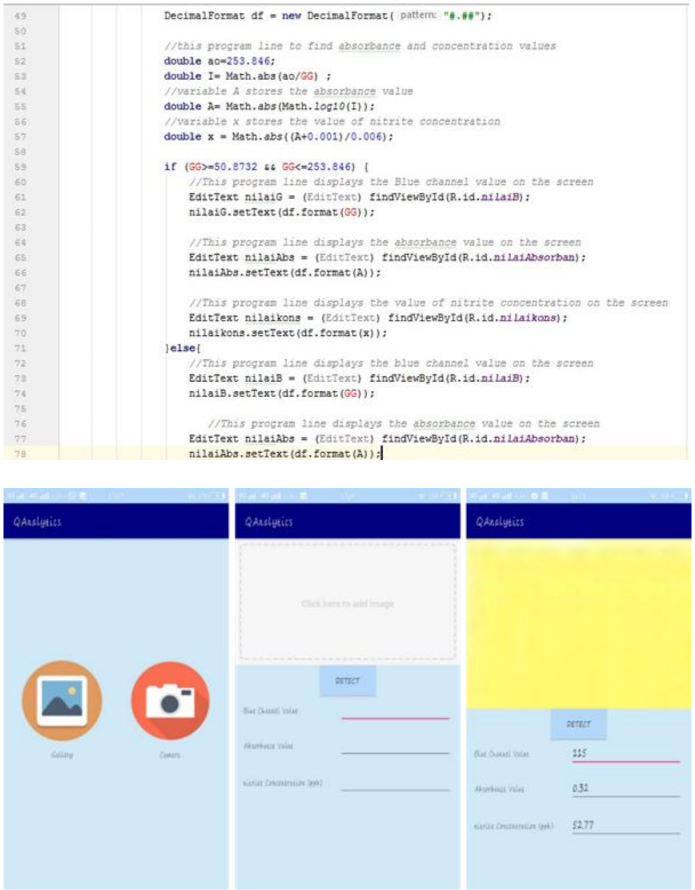


Fig. 1. Example of coding for 'QAnalytics' smartphone application (upper panel), and the appearance of application interface to detect nitrite concentration in samples (below panel).

3.2. Analytical performance and application to real samples

The proposed digital image and smartphone-based colorimetry method give a good linear correlation as shown in Figure 2. The regression equation is y = 0.006x with correlation coefficient of 0.9999. The detection limit of the method was 3.2 ppb that was computed from 3 times the standard deviation (σ) of 10 blank measurements, divided by the slope of the calibration curve (3σ of the blank/slope).

Compared to previously published reports shown in Table 1, it is clear that the proposed method has several advantages such as better detection limit, fewer chemicals and instruments usage in performing the analysis, portability, and simplicity. Although the detection limit of this work is higher than some of the method using common analytical instruments (i.e. fluorescence, GC – MS), the analytical performance of the method (i.e. LOD = 3.2 ppb) is sufficient to detect the nitrite contamination in drinking water as the permissible nitrite concentration set by WHO is 0.2 ppm.

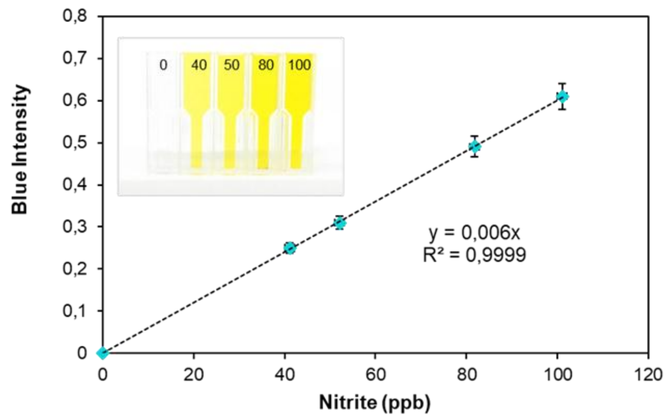


Fig. 2. Calibration curve using digital image-based (DIB) colorimetry attached in a smartphone with Blue intensity as the complementary color of yellow nitrite compound. Error bars were computed from 3 replicates. The inset shows a photograph of nitrite standards in cuvettes.

Table 1. Limit of detection (LOD) for nitrite analysis using the proposed protocol compared to the previously reported method.

Method	Chromogenic reagent	LOD	Ref.
Spectrophotometry	Sulfanilic acid	0.93 ug/mL (930 ppb)	[9]
Spectrophotometry	p-aminobenzoic acid	0.52 uM (24 ppb)	[8]
DIB colorimetry – scanner	Sulfanilamide / Griess	1 uM (46 ppb)	[15]
DIB colorimetry – scanner	Sulfanilamide / Griess	5.6 uM (258 ppb)	[16]
DIB colorimetry – scanner	Tetrahydroquinoline	1–3 ng/mL (1-3 ppb)	[14]
DIB colorimetry – smartphone	Sulfanilamide / Griess	0.52 mg/L (520 ppb)	[17]
DIB colorimetry – smartphone	Tetrazine	1.3 uM (60 ppb)	[18]
CE – Fluorescence	-	0.6 nM (0.03 ppb)	[11]
HPLC – Fluorescence	-	10 pmol/mL (0.46 ppb)	[12]
LC – Fluorescence	-	0.29 pg/mL (0.29 ppt)	[10]
GC – MS	-	0.02 pg/mL (0.02 ppt)	[10]
DIB colorimetry – smartphone	p-aminobenzoic acid	0.07 uM (3.2 ppb)	This work

In order to validate the approach, recovery experiments from spiked samples was done using the proposed method, with spectrophotometry as a reference method. Bangkahulu river water from Bengkulu city was chosen as the target samples. Using the proposed method, quantitative recoveries from 98 to 103% were achieved (Table 2). The accuracy of the proposed method, evaluated as the % error of the digital image coupled with a smartphone-based colorimetry, was less than 2.5%. While the precision, evaluated as the % RSD (relative standard deviation) from replicate analyses, was better than 4.9%. Based on these analytical performances, we concluded that accuracy and precision were satisfactory.

Table 2. Recovery experiments from spiked samples (n = 3).

Sample	Added (ppb)	DIB Colorimetry – Smartphone		Spectrophotometry (ppb)
		Found (ppb)	Recovery (%)	
Upstream river water	0	4.1 ± 0.2	-	4.2
	50	53 ± 2	98	54
	100	105 ± 5	101	104
Downstream river water	0	4.5 ± 0.1	-	4.6
	50	56 ± 2	103	55
	100	106 ± 5	101	105

4 Conclusion

In summary, we reported for the first time a sensitive and selective nitrite quantification by means of digital image-based colorimetry attach within a smartphone. Under the optimized conditions, detection limit was 3.2 ppb with satisfactory accuracy and precision. The proposed method has several advantages over previously reported methods, such as sensitivity, selectivity, simplicity and portability. It is expected that this protocol will provide a low-cost alternative to analyze nitrite ions in remote areas with limited resources.

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