Development of Microfluidic Paper-Based Analytical Devices for Simultaneous Colorimetric Ammonium and Nitrate Detection

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Abstract

The frequency of terrorist attacks involving Improvised Explosive Devices (IEDs) is expected to continue increasing. Ammonium nitrate fuel oil (ANFO) is the preferred main charge in IEDs due to its low cost and wide availability. Hence, there is a pressing need to develop an ammonium nitrate detection method that is easy to use, cost-efficient, fast, and reliable. This research developed the µPADs as viable devices for detecting ammonium nitrate in the field alongside a mobile phone camera. In this study, a two-lane µPAD was developed to detect both ammonium and nitrate ions in an ammonium nitrate solution. The purpose of this research was to develop a simple and low-cost colorimetric method for simultaneous ammonium and nitrate ion detection using microfluidic paper-based analytical devices (µPADs) fabricated by the wax-screen printing technique. The two-channel µPADs were designed and fabricated. The first channel was used for ammonium detection using Nessler's reagent coated on the detection at the end of the channel. The second channel was used for nitrate detection using a Griess reagent. By simply dropping the sample at the center of µPADs, the developed µPADs were able to detect both ammonium and nitrate in a short time (only 4 minutes). The colored product on the µPAD (yellowish-brown and pink color for ammonium and nitrate, respectively) was then captured with a camera on a smartphone under a commercial controlled light box and its color intensity was obtained by using ImageJ software. Using the NH4NO3 concentration in the range of 100 – 800 ppm, under optimum conditions, there was a good linear relationship (r2 = 0.9961 for ammonium concentration range of 200-800 ppm and r2 = 0.9894 for nitrate concentration range of 100-800 ppm) and the detection limit was 48.760 and 81.966 ppm for ammonium and nitrate, respectively.

Keywords: Microfluidic Paper-based Analytical Devices, Colorimetric Detection, Ammonium and Nitrate Detection

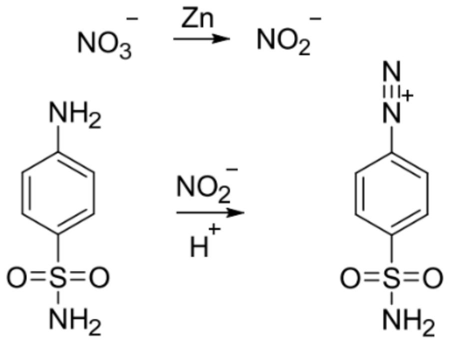
1. Introduction

Terrorist attacks using Improvised Explosive Devices (IEDs) are likely to increase continuously [1]. IEDs consist of various components that include an initiator, switch, explosive material, power source, and a container [2]. Numerous materials that are commonly available, such as fertilizer, gunpowder, and hydrogen peroxide, can be employed as explosive substances in IEDs. Fuel and an oxidizer, which supplies the oxygen required to maintain the reaction, are required to produce explosive materials. The combination of fuel oil (acting as the fuel source) and ammonium nitrate ($NH4NO3$, acting as the oxidizer), known as ANFO (ammonium nitrate fuel oil), is frequently used as a main charge in IEDs due to its low cost and wide availability [2, 3]. Ammonium nitrate is a potent oxidant widely used as fertilizer and an industrial explosive. Due to its colorless, odorless crystal structure, identification requires various analysis methods [4]. The efficient identification of explosive ingredients is a crucial aspect of forensic science. Including information on explosive chemicals aids the inquiry in finding the possibility of the origin of the explosives [5]. Ammonium nitrate detection methods commonly involve instrumental analysis, including electrospray ionization mass spectrometer (ESI-MS) and inductively coupled plasma–mass spectrometry (ICP–MS) [6, 7]. These instrument-based techniques have great sensitivity and accuracy, but they are expensive, large, non-portable, and time-consuming. The lengthy process required for this complicated procedure delays the notification of on-site personnel. Therefore, it is essential to develop an ammonium nitrate detection technique that is easy to use, cost-effective, rapid, and reliable.

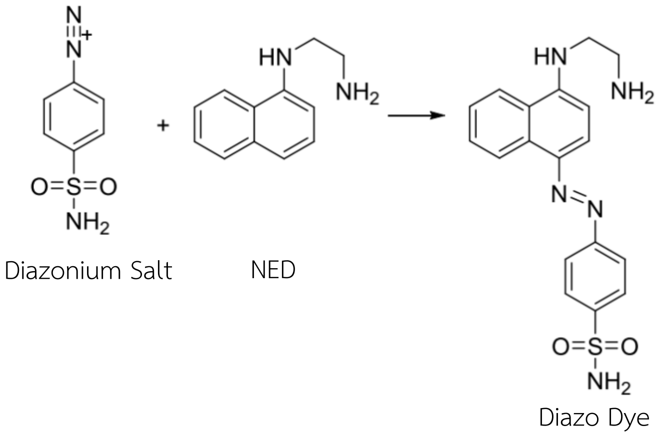
Microfluidic paper-based analytical devices (μPADs) are devices fabricated from paper with microchannels created by incorporating hydrophilic and hydrophobic regions to facilitate fluid flow. They have become an increasingly attractive substrate for on-site testing because of their affordability, independence from other instruments, portability, low consumption of reagents and samples, and ease and rapidity of fabrication [8]. Most μPADs use filter paper as a hydrophilic substrate, with a hydrophobic barrier made using many different techniques. Hydrophilic fluid cannot pass through the hydrophobic barrier; therefore, it is directed into the channels through capillary action. There are several techniques for the fabrication of μPADs including wax printing, photolithography, flexographic printing, inkjet printing, plasma treatment, laser treatment, wet etching, screen-printing, and wax screen-printing [9, 10]. The most widely used analytical detection method for μPADs is colorimetry, which requires specific reagents that change color to correlate with the analyte concentration. The relationship between color intensity and analyte concentration can be established more easily through technology using smartphone reporting methodologies [11-13].

Additionally, employing an electronic device (such as a digital camera, smartphone, or scanner) coupled with image-processing software (such as ImageJ, Photoshop, etc.) can generate qualitative and quantitative results [14, 15]. Recently, various studies have reported the development of μPADs for medical diagnostics, environmental monitoring, and explosive detection [8-10, 16].

High-explosives and military explosives have also been tested using colorimetric methods along with microfluidic devices [13]. In 2015, Peters and colleagues developed μPADs by designing a five-lane μPAD and printing wax ink on chromatography paper for the detection of inorganic explosives, such as ammonium nitrate, urea nitrate, and black powder [16]. Colorimetric-based tests for ammonium nitrate detection are divided into two parts: ammonium ion (NH4+) and nitrate ion (NO3-). Typically, Nessler's reagent is employed for the identification of ammonium ions. This reagent consists of a combination of mercury(II) chloride (HgCl2) or mercury(II) iodide (HgI2) and potassium iodide within an alkaline solution. This mixture yields potassium tetraiodomercurate(II) (K2[HgI4]). When Nessler's reagent interacts with ammonia resulting from the reduction of ammonium, it generates a yellow-brown color complex [16, 17]. The identification of nitrate ions can be conducted using the color reaction of the Griess reagent. This approach is most commonly employed for detecting nitrite and nitrate [18]. For the detection of nitrate, it is necessary to first reduce nitrate to nitrite using an appropriate reductant (such as Zn or Cd) before proceeding to the diazotization step in the testing process based on the Griess reaction. Subsequently, nitrite reacts with sulfanilamide (or sulfanilic acid) under acidic conditions, yielding a diazonium salt. This salt further combines with an electron-rich coupling agent, such as α-naphthylamine or N-(1-naphthyl) ethylenediamine (NED), resulting in the formation of a colored azo dye [19-21]. The Griess reaction for the detection of nitrate follows the equations below [19].



Sulfanilamide Diazonium Salt



The objective of this research was to develop a two-lane μPAD capable of simultaneously detecting both ammonium ion and nitrate ion in ammonium nitrate solution, with the goal of providing preliminary data for the field detection of ANFO. Wax screen-printing was used for fabricating μPADs because it is a low-cost, simple, and rapid method [9]. This detection process included the utilization of the color reaction of Nessler's reagent and Griess reagent, a digital image analysis approach, and the ImageJ program. The optimal time for measurement of the color intensity was also studied. The concept of this research is illustrated in Fig. 1.

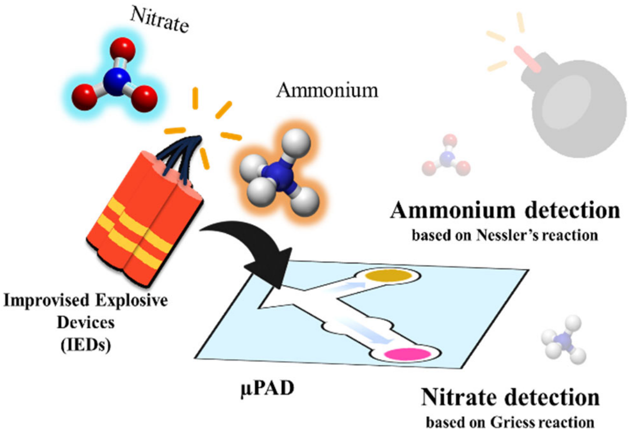


Fig. 1 The concept of ammonium nitrate detection in this study.

2. Materials and Methods

2.1 Chemicals and Apparatus

Nessler's reagent was prepared by dissolving 5.0 g of potassium iodide (Ajax Finechem, Australia) in 5 mL of deionized water and then adding saturated mercuric iodide solution until a faint precipitate was formed. Finally, 40 mL of 50% w/w potassium hydroxide was added and the volume was adjusted with deionized water to 100 mL using a volumetric flask [22]. The solution was decanted after being allowed to stand overnight away from bright light. Griess reagent consisted of three components as follows: (1) 5% zinc suspension; (2) 50 mM sulfanilic acid in 1.5 M citric acid; and (3) 10 mM N-(1-Naphthyl)-ethylenediamine dihydrochloride (NED.Cl) solution [16, 18]. The 5% zinc suspension was prepared by mixing 500 mg of zinc powder (Kemaus, Australia) in 10 mL of deionized water. The 50 mM sulfanilic acid in 1.5 M citric acid was prepared by dissolving 86.5 mg of sulfanilic acid (TCI, Japan) in 78.8 g of citric acid (TCI, Japan) dissolved in 10 mL of deionized water. The 10 mM N-(1-Naphthyl)-ethylenediamine dihydrochloride (NED.Cl) solution was prepared by dissolving 27.2 mg of NED.Cl (TCI, Japan) in 10 mL of deionized water. Various concentrations of ammonium nitrate standard solutions were prepared by dissolving ammonium nitrate (Sigma-Aldrich, USA) in deionized water. All used chemicals and reagents were analytical grade. Deionized water was used for the preparation of all aqueous solutions.

The LED light in the commercial control light box (96 high-display lamp beads, 10W) in this study is a plastic box (36 cm × 36 cm × 24 cm) purchased from Dongguan Puluz Technology Limited (China).

2.2 Design and fabrication of the μPAD

In this work, a simple wax screen-printing technique was used for the μPADs fabrication. The patterned screen was designed using the Notes Writer application. The design of μPADs is shown in Fig. 2. In the μPADs pattern, there was a 7 mm diameter for both the detection and reduction zone. Additionally, the transport lane was set at 4 x 7 mm. A triangular-shape sample zone was created to facilitate the flow of sample solution from the sample region to each detection zone simultaneously. The sample zone of the μPADs was designed as an equilateral triangle with a side length of 0.7 cm. The patterned screen was made from 64-mesh polyester fabric on a wooden frame. For the fabrication step, first, Whatman filter paper no.1 was placed under the patterned screen. Then, solid wax (hydrophobic substrate) was rubbed through the designed area on a patterned screen and then the excess of wax was removed from the pattern with a squeegee. To make the hydrophobic barrier on paper, a hot hair dryer was used to melt the wax. After 15 seconds of blowing, the μPAD was obtained. A 2 μL volume of each colorimetric reagents was spotted onto the μPADs which was sufficient to develop color across the reaction zone without any leakage over the hydrophobic barrier.

Initial testing with these μPADs involved selecting reagents from a literature review [16, 18-19], as many colorimetric methods use strong acids that may damage the paper. A weak acid was then used in the nitrate test, still producing a detectable color change. The main parameters of the proposed μPADs, including the deposited volume of Nessler’s reagent and the Griess reagent (zinc suspension, sulfanilic acid in citric acid, NED.Cl solution), the citric acid concentrations in the Griess test, and the sample volumes, were optimized for sensitivity and clear color observation by the naked eye. The range of each optimized parameter is shown in Table 1.

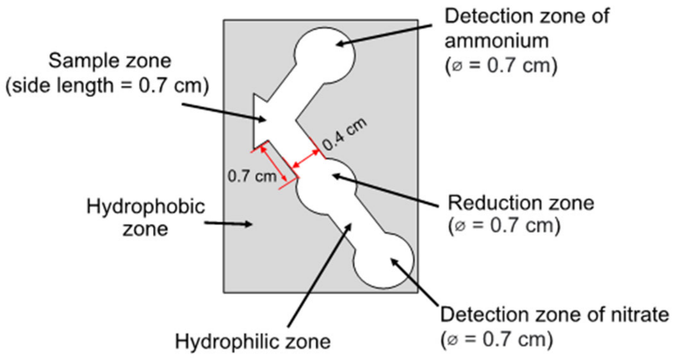


Fig. 2 The design and dimension of µPAD for simultaneous ammonium and nitrate detection.

Table 1. Summary of µPAD parameters investigated in this study.

Parameter	Range tested	Optimum value
Deposited volume of Nessler's reagent (µL)	1.0 - 5.0	2.0
Deposited volume of Griess reagent		
- 5% Zinc suspension (µL)	1.0 - 5.0	2.0
- 50 mM sulfanilic acid in 1.5 M citric acid (µL)	1.0 - 5.0	2.0
- 10 mM of NED.Cl (µL)	1.0 - 5.0	2.0
Concentrations of citric acid for preparation of sulfanilic acid (M)	0.3 - 1.5	1.5
Ammonium nitrate standard solution volume (µL)	10 - 30	20

2.3 Simultaneous colorimetric detection of ammonium and nitrate using fabricated µPAD

As shown in Fig. 3, for the detection of ammonium, 2 µL of Nessler's reagent was pipetted onto the detection zone of the first channel. To detect nitrate with Griess reagent, on the second channel, three nitrate detection reagents were applied including 2 µL of each 5% Zinc suspension and 50 mM sulfanilic acid dissolved in citric acid on the reduction zone. Then, 2 µL of 10 mM of N-(1-Naphthyl)-ethylenediamine dihydrochloride (NED.Cl) was dropped on the detection zone. After drying for a minute, the µPAD is ready for detection.

In the detection step, 20 µL of standard ammonium nitrate solution was dropped into the sample zone and flowed through each channel to react with the coated reagent, resulting in a color change in the detection zone. The detection zone of the ammonium reaction showed a yellowish-brown complex, while the detection zone of the nitrate reaction showed a pink-colored product. The images of the colored products in the detection zones were then captured using a camera on a smartphone (OPPO A74, China). Subsequently, the RGB intensity values of these images were obtained utilizing ImageJ software. The average color intensity for each detection zone at various times was calculated using 3 replicate measurements. The limit of detection (LOD) and limit of quantification (LOQ) were calculated by finding the analyte concentration corresponding to the intensity values YLOD and YLOQ on the calibration curve using the Miller method [23].

The number of photons of light reflected from the color of the product on the μPADs into the RGB filter (Red, Green, Blue filter) corresponds to the color intensity measured by the ImageJ software. Since the blank μPAD is white, all its color intensities are close to the value of 255. The color intensity of the product resulting from the reaction between reagent and ammonium nitrate solution was measured and compared to the color intensity of a blank μPAD. The red, green, and blue intensities of the blank μPAD are subtracted from the measured intensities of the color product, resulting in the gray intensity in the red channel (R0-R), the green channel (G0-G), and the blue channel (B0-B), respectively. To plot the calibration curve, the gray intensity in the blue channel (B0-B) and gray intensity in the green channel (G0-G) for ammonium and nitrate detection, respectively, were then used. While B0 and G0 are gray intensity in the blue and green channels without analyte.

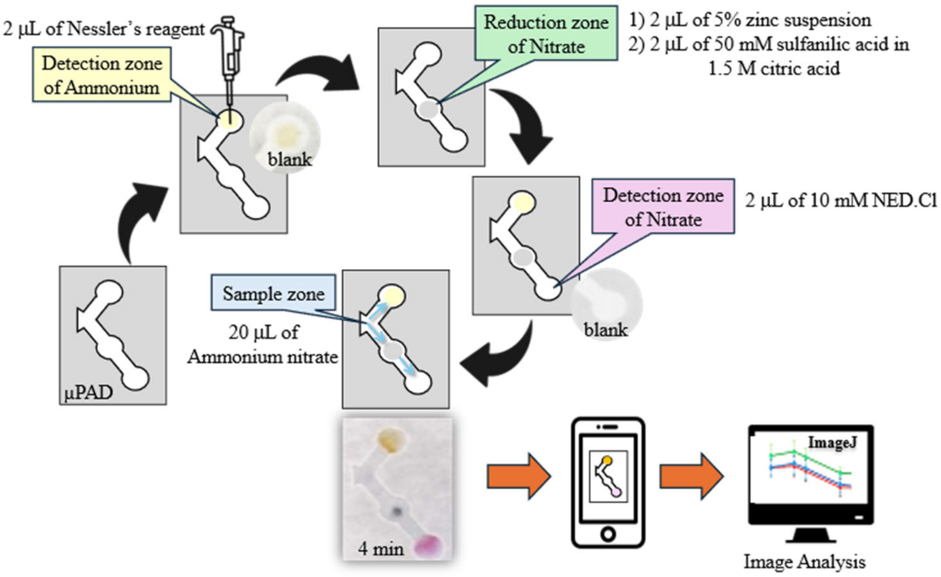


Fig. 3 Schematic illustration of colorimetric detection of ammonium and nitrate on the μPAD.

3. Results and Discussion

3.1 Optimization of incubation time

The optimal time for measurement of the color intensity was determined after applying the ammonium nitrate solution with respect to cleared colors observed by the naked eyes and the resulting colors appeared in the detection zone only. To analyze the μPAD, an initial detector is the observation with the naked eye, which is the simplest and most cost-effective method because it does not require any additional equipment. A smartphone is another common device that offers excellent imaging capabilities and ensures the sharpness of digital images.

A 2 µL of Nessler's reagent was applied to the detection zone in ammonium testing. For the detection of nitrate, a sequential 3-step test was chosen based on the Griess test. At the reduction zone, the nitrate sample was reduced by zinc powder. Following reduction to nitrite, the sample reacts with an acid catalyst and sulfanilic acid to form diazonium salt at this reduction zone. The diazonium salt continued to travel up the hydrophilic lane to combine with a coupling agent (NED.Cl) to produce a brightly colored diazo dye at the detection zone. From the experiment, the steps of Griess reagent for nitrate detection on µPADs are detailed as follows: (1) 2 µL of 5% zinc suspension was spotted on the reduction zone to reduce the nitrate to nitrite; (2) 2 µL of 50 mM sulfanilic acid in 1.5 M citric acid was added on the reduction zone after depositing Zn to form diazonium salt; and (3) 2 µL of 10 mM NED.Cl was spotted on detection zone (The diazonium salt continued to combine with a coupling agent to produce a brightly colored diazo dye).

For incubation time, the µPAD facilitates the simultaneous detection of both ammonium and nitrate in separate channels, providing results in a short time with strong color intensity. A 20 µL of 700 ppm ammonium nitrate solution was used as the sample and spotted at the sample zone to investigate the color development time and the RGB intensity of the color product on the µPADs. After applying the sample for 2, 4, 5, 8, and 11 minutes, the color development times were recorded and measured the RGB intensity using the ImageJ program. The digital images of the products on the µPAD that appeared at different times are shown in Fig. 4.

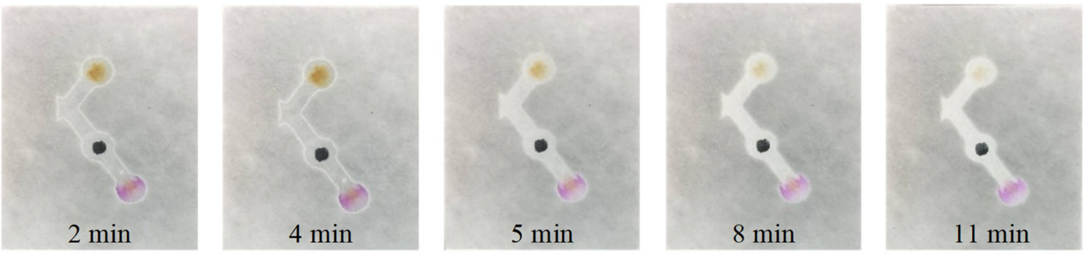


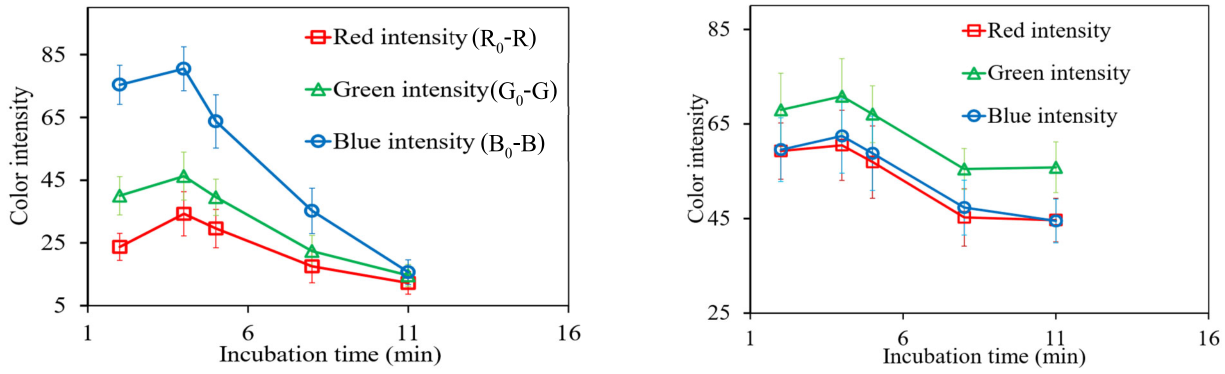
Fig. 4 The digital images after applying ammonium nitrate on the µPAD at different times.

The reaction of Nessler's reagent and ammonium generated a yellowish-brown product that formed on the µPAD, which had the highest value of the blue intensity (B0-B). Similarly, the color intensity measurements of the pink product resulting from the reaction between Griess reagent and nitrate on the µPAD shows the highest green intensity (G0-G). These results are shown in Fig. 5 and Fig. 6. Based on the data, it was observed that the blue intensity was most sensitive for ammonium detection, and the green intensity exhibited the highest sensitivity for nitrate detection. The maximum color intensity in the simultaneous detection of ammonium and nitrate occurred at 4 minutes, followed by a decrease in intensity between 4 and 11 minutes. The optimal incubation time for a measurement was selected at 4 minutes.

3.2 Analytical performance using the μPADs for colorimetric detection of ammonium nitrate

To study the relationship between the concentration of ammonium nitrate and the color intensity of the product on the μPADs, a range of ammonium nitrate solution concentrations from 100 to 1600 ppm were used in this study. After applying ammonium nitrate solution, the digital photos of the color products on the μPADs were captured at 4 minutes using the OPPO A74 smartphone camera.

From the experiment, the color product changes from yellow to yellowish-brown for ammonium detection and changes from colorless to pink for nitrate detection. The results in Fig. 7 indicated the measurement of blue intensity derived from the color product on the μPADs in the ammonium test. The linear relationship between blue intensity values and ammonium concentration for quantitative analysis, was represented by the equation y = 0.0386x + 54.22 with a correlation coefficient (r2) of 0.9961, was determined in the ammonium concentration range of 200 to 800 ppm with limit of detection (LOD) and quantification (LOQ) of 48.760 ppm and 162.533 ppm, respectively. Similarly, the researchers conducted measurements of green intensity obtained from colorless to the pink product on the μPADs generated during the nitrate test. Fig. 8 shows that the green intensity values exhibited a linear correlation with nitrate concentration in the range of 100 to 800 ppm, which is equivalent to ammonium. This led to constructing a linear calibration curve by plotting the resulting green intensity as a function of nitrate concentration for quantitative analysis (y = 0.0542x + 37.98, r2 = 0.9894) with LOD and LOQ of 81.966 ppm and 273.219 ppm, respectively. The findings demonstrate that the obtained LOD is lower than those reported in earlier studies on ammonium and nitrate testing of high explosives using μPAD [16].



(A) (B)

Fig. 5 Relationship between the color development time and the color intensity of the product for (A) ammonium detection (B) nitrate detection.

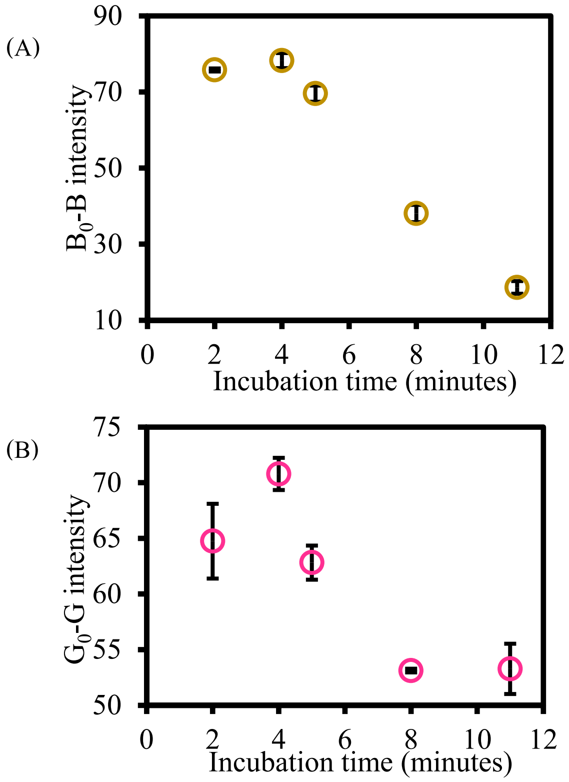


Fig. 6 Optimum incubation time for simultaneous ammonium and nitrate detection on the μPADs for (A) ammonium detection (B) nitrate detection. The error bars represent the standard deviation (SD) of three repeated measurements (n = 3).

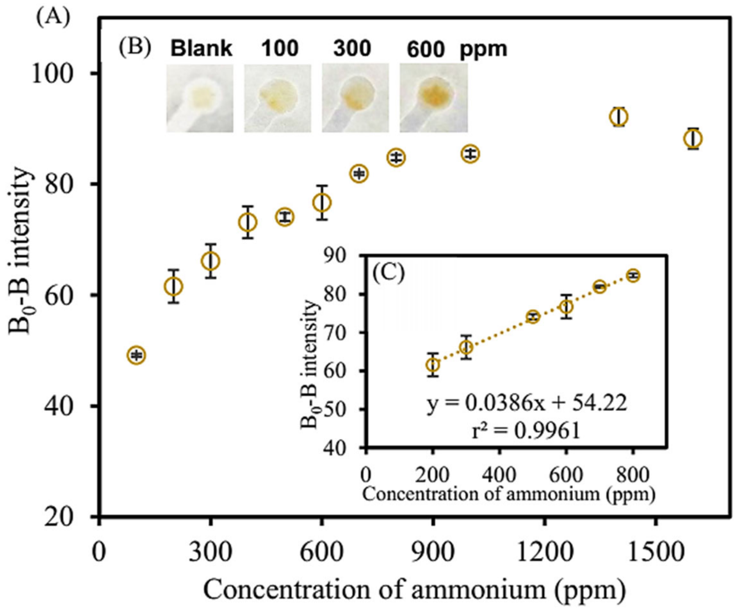


Fig. 7 (A) Linear calibration plot for ammonium detection inset (B) pictures of the color change of μPAD and (C) calibration concentration of ammonium.

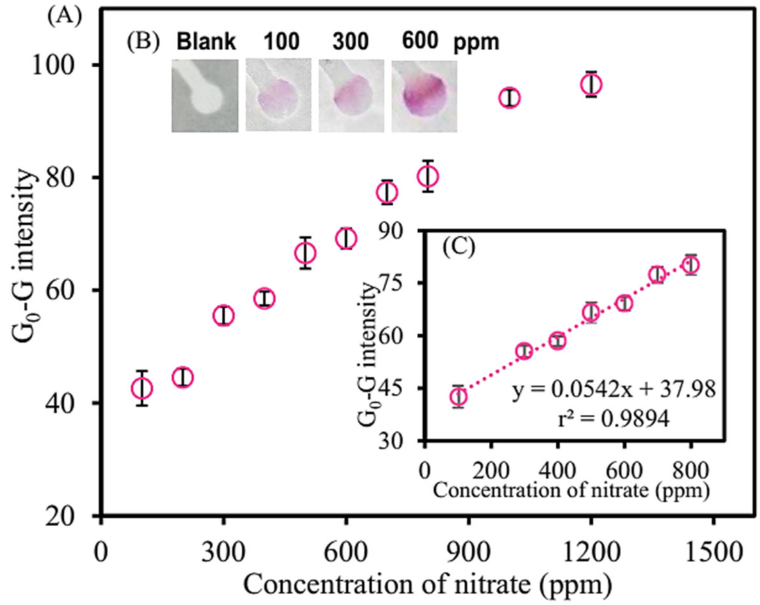


Fig. 8 (A) Linear calibration plot for nitrate detection inset (B) pictures of the color change of μPAD and (C) calibration concentration of nitrate.

4. Conclusions

This research developed the μPADs as viable devices for detecting ammonium nitrate in the field alongside a mobile phone camera. Additionally, it is feasible to integrate the μPADs with the program to create a device for quantifying ammonium nitrate levels in samples. The advantage of this research is that shortly after the sample is applied, both ammonium and nitrate can be detected through visual inspection and analysis using a smartphone camera. These μPADs will provide law enforcement for analyzing unknown suspected ammonium nitrate in ANFO. Furthermore, this method is simple, low cost, portable and rapid for on-site analysis of explosives without requiring elaborate laboratory equipment.

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