Simple Colorimetric Sensor for Trinitrotoluene Testing

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Abstract. A simple operating colorimetric sensor for trinitrotoluene (TNT) determination using a commercial scanner as a captured image was designed. The sensor is based on the chemical reaction between TNT and sodium hydroxide reagent to produce the color change within 96 well plates, which observed finally, recorded using a commercial scanner. The intensity of the color change increased with increase in TNT concentration and could easily quantify the concentration of TNT by digital image analysis using the Image J free software. Under optimum conditions, the sensor provided a linear dynamic range between 0.20 and 1.00 mg mL⁻¹ (r = 0.9921) with a limit of detection of 0.10± 0.01 mg mL⁻¹. The relative standard deviation for eight experiments for the sensitivity was 3.8%. When applied for the analysis of TNT in two soil extract samples, the concentrations were found to be non-detectable to 0.26±0.04 mg mL⁻¹. The obtained recovery values (93-95%) were acceptable for soil samples tested.

1. Introduction

2,4,6-trinitrotoluene (TNT) is one of the most widely used explosive compounds for the military, terrorist activities and mining. TNT is also highly toxic to many organisms because it could cause liver damages and many other health problems [1]. Therefore, the detection of TNT has attracted much attention due to these environmental and health related concerns. On the other hand, the identification of its post explosive residues in forensic chemistry is also needed for use as a guideline to help narrow any investigation associated with securities [2].

A wide range of methods including gas or liquid chromatographic analysis [3-4], electrochemical methods [5] spectrophotometry [6] and colorimetric sensor have been applied for the quantitative analysis of TNT. Among these, the colorimetric sensor is an interesting approach due to its fast response, simplicity, low cost, and easy preparation of standard solutions. Moreover, the color change can be easily detected by naked eyes. A number of commercial chemical kits such as DROPEXPlus and EXPRAY have also been developed for TNT detection. However, these methods could be used only for qualitative analysis.
For a quantitative analysis and off-site detection, a novel quantitative analytical methodology based on digital image analysis of the photographs of the reaction test has been reported. The digital image was obtained by pocket camera [7] or mobile phone camera [8]. The reflected light from objects passes through and is detected by three RGB filters (Red Green and Blue) by using the Adobe Photoshop software [8].

The aim of this work is to demonstrate the application of a simple, rapid, cost-effective and quantitative analysis colorimetric sensor for TNT determination using sodium hydroxide (NaOH) as a tested reagent. The color change was recorded using commercial document scanner. The intensity of the red-violet color change will be increased after a reaction. This method can quantify the concentration of TNT by scanner image analysis using the Image J free software and can be applied for the detection of TNT contaminated soils.

2. Experimental

2.1. Materials and apparatus

2,4,6-Trinitrotoluene (TNT) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile was purchased from RCI Labscan (Bangkok, Thailand). Sodium phosphate diabasic AR and sodium dihydrogen orthophosphate AR were obtained from Lobachemie (Mumbai, India). The digital image was captured using the commercial scanner (Epson Perfection™ V39, Indonesia).

2.2 TNT detection

TNT in acetonitrile was used as the stock solution. The stock solution was further diluted with acetonitrile to appropriate concentrations in the range of 0.10 to 1.00 mg mL$^{-1}$ to produce a range of TNT standards. A TNT color test was performed using NaOH as the reagent. One hundred microliters of each TNT standard solution was pipetted to a 96 well plate and two hundreds of 0.30 M NaOH in phosphate buffer pH 7.00 was added to obtain a red–violet product. The resultant colors were captured five minutes after color development using the commercial document scanner. Each concentration of prepared standards was repeated four times and each resultant color was recorded three times using the commercial scanner. The images were transferred to a computer (Microsoft Windows 7). The average color intensity of Red, Green, and Blue of each color product in each sensor was obtained using the “Crop” tool and the “Histogram” in Image J free software and transferred the data into a Microsoft Office Excel 2010 spreadsheet for subsequent data analysis. A calibration graph was prepared for each color. The limit of detection was calculated using standard methods [9] and precision was expressed as a percentage of the relative standard deviation of the RGB values for each color from the three analyzed images. The schematic presentation for TNT detection procedure is shown in Figure 1.
The initial detection conditions were 10 mins of reaction time, 150 μL of 0.6 M NaOH in phosphate buffer pH 8.00 and 150μL of TNT in acetonitrile. The detection response was the intensity of the color change of RGB values. All of these operating conditions were optimized to obtain the highest intensity color change of the colorimetric sensor as follows:

2.2.1 Reaction time
Optimization of standing time of the reaction between TNT and the tested reagent is necessary for the completion of the TNT reaction in basic medium. Hence, the effect of reaction time was studied in the range of 1-20 mins. At the optimum reaction time, the highest intensity color change was then selected for use in the next experiments.

2.2.2 Concentration of NaOH
The initial results showed that the maximum intensity color change of TNT detection was obtained in the alkaline medium due to the formation of Meisenheimer anion. Therefore, to succeed suitable basic media, the effect of different concentration of NaOH in the range of 0.15-1.20 M was investigated. Then at the optimum concentration of NaOH with the highest intensity color change was chosen.

2.2.3 The volume of test NaOH
The volume of test NaOH reagent was optimized to determine the volume required to produce the darkest color reaction for the least concentrated analyte solution. Therefore, to investigate this objective, the volume of the tested NaOH in the range of 100-250 μL was obtained.
2.3 Analytical performances

2.3.1 Linear dynamic range and detection limit
Under optimum conditions, the linear dynamic range was investigated between 0.10 and 1.00 mg mL$^{-1}$. The limit of detection was evaluated from the equation $X_L = X_{bl} + 3(S_{bl})$ where $X_L$ was the signal at the limit of detection, $X_{bl}$ and $S_{bl}$ are the mean and the standard deviations of the blank response [9].

2.3.2 Repeatability
Repeatability was studied by comparing the sensitivity (slope of the calibration curves) of eight different experiments, and the relative standard deviation was calculated.

2.3.3 Soil samples analysis
A soil sample (1 g) was extracted with 2.5 mL of ethyl acetate and sonicated for 15 mins before filtering using Whatman No. 1 filter paper. The supernatant was spiked with a TNT standard solution to investigate the effect of the color of the soil filtrate on the TNT presumptive test color derived from the test. The extracted solution was analyzed according to the procedures described in the Section of 2.2.

3. Results

3.1 Optimization
No color change was observed for the reagent in the absence of TNT (blank). On the other hand, red-violet products were obtained from the NaOH test for the presence of TNT (Figure 2a). The color change is because, in the basic medium, the TNT reacts with NaOH to give the “Meisenheimer anion” which is a color product [10-11]. Therefore, it can be said that the color change was caused by the TNT complex from colorless to red-violet color (Figure 2b). The color change also can be easily detected by naked eyes. In summary, the optimum detection conditions were reported in Table 1.

| Parameters                        | Studied ranges         | Optimal values |
|-----------------------------------|------------------------|----------------|
| Reaction time                     | 1, 5, 10, 15 and 20 mins| 5 mins        |
| Concentration of test NaOH        | 0.15, 0.30, 0.60 and 1.20 M | 0.30 M        |
| The volume of test NaOH           | 100, 150, 200 and 250µL | 200 µL         |

3.2 Color test of TNT
Under the optimum conditions, the color change with the increasing TNT concentration was studied in the range of 0.10 to 2.00 mg mL$^{-1}$. The resulted series color change was then recorded using the commercial scanner, followed by analysis of their intensity of the color change using free software of Image J to get RGB profile (Figure 1 example histogram of channel green). The relation between the intensity of color the change and TNT concentration was selected from the three colors of Red, Green or Blue with the highest sensitivity and best regression coefficient. The result showed the best-reducing color intensity was green, blue and red, respectively (Figure 2c). Then the channel green was used for the prepared soil extract analysis. The linear dynamic range was 0.20 to 1.00 mg mL$^{-1}$ and the detection limit was determined and found to be 0.10±0.01 mg mL$^{-1}$. 
Figure 2. (a) The example color changes with the TNT concentration in a 96-well plate captured by the scanner. (b) The reaction of TNT in basic medium and (c) Calibration curves of TNT colorimetric sensor in a series TNT concentration (0.20 – 1.00 mg mL\(^{-1}\)) of (\(\Delta\)) channel red, (o) green and (◻) blue.
3.3 Repeatability
The repeatability was investigated through the sensitivity of eight experiments. Between the eight experiments, the relative standard deviations (RSDs) of 3.8% (Figure 3), indicated a good repeatability of the developed colorimetric sensor. This RSD was within the acceptance criteria (less than 8% RSD in the ppm level) [12].

![The relative standard deviation (RSD) of 3.8%](image)

Figure 3. Repeatability of the sensitivities of the eight different experiments

3.4 Quantification of TNT in soil
The proposed colorimetric sensor was applied for the detection of TNT in prepared soil samples. Table 2 summarizes the results obtained for the two samples analyzed. TNT was detectable with the contents of non-detectable in sample No.1 and 0.26 ± 0.04 mg mL\(^{-1}\) in another sample. For validation of the proposed method, TNT standard solution was spiked into soil extract (0.50 mg mL\(^{-1}\)) as previously described. The obtained recovery values (93-95%) were acceptable for soil samples tested. This recovery value was within the acceptance criteria (between 90-107% in the ppm level) [12].

| Soil sample No. | Added (mg mL\(^{-1}\)) | Found* (mg mL\(^{-1}\)) | Recovery (%) |
|-----------------|-------------------------|--------------------------|--------------|
| 1               | 0.00                    | ND                       | -            |
|                 | 0.50                    | 0.48±0.02                | 95±3         |
| 2               | 0.00                    | 0.26±0.04                | -            |
|                 | 0.50                    | 0.72±0.02                | 93±4         |

* average ± standard deviation (n =3)
ND = non-detectable

4. Conclusion
In this work a simple and rapid (5 mins) was applied for the TNT testing based on the use of NaOH as a tested reagent and the used of the document scanner as a capturing digital image. This colorimetric sensor can be easily observed by naked-eyes. This work also provided a good repeatability and recoveries. Moreover, it was successfully applied for the analysis of TNT in soil extract samples. This can be used to indicate the quality of soil samples which is crucial for agricultural, environmental and health safety. Also this colorimetric sensor could be identified the TNT in explosive residues in...
forensic chemistry to be used as a guideline to help narrow any investigation associated with securities. This commercial scanner detector together with the image J software program could certainly be easily extended to different analytes using different designed colorimetric sensor reaction.

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Acknowledgements
This research was supported by the Nation Research Council of Thailand. Partial support from and Faculty of Science and Technology, Princess of Naradhiwas University, Thailand are gratefully acknowledged. Thanks also to Miss Tshering Pelden for assistance with the English.