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## **A cost effective hydrogel test kit for pre and post blast trinitrotoluene**

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## ABSTRACT

A cost effective hydrogel test kit was successfully developed for the detection of pre- and post-blast trinitrotoluene (TNT). A polyvinyl alcohol (PVA) hydrogel matrix was used to entrap the potassium hydroxide (KOH) colourimetric reagent. The easily portable test kit was fabricated *in-situ* in a small tube to which the sample could be added directly. The test kit was used in conjunction with digital image colourimetry (DIC) to demonstrate the rapid quantitative analysis of TNT in a test soil sample. The built-in digital camera of an iPhone was used to capture digital images of the colourimetric products from the test kit. Red-Green-Blue (RGB) colour data from the digital images of TNT standard solutions were used to establish a calibration graph. The validation of the DIC method indicated excellent inter day precision (0.12-3.60%RSD) and accuracy (93-108% relative accuracy). Post-blast soil samples containing TNT were analysed using the test kit and were in good agreement with spectrophotometric analysis. The intensity of the RGB data from the TNT complex deviated by +6.3%, +5.1%, and -4.9% after storage of the test kits in a freezer for 3 months. The test kit was also reusable for up to 12 times with only -5.4%, +0.3%, and +4.0% deviations. The hydrogel test kit was applied in the detection of trace explosive residues at the scene of the recent Bangkok bombing at the Ratchaprasong intersection and produced positive results for TNT demonstrating its operational field application as a rapid and cost effective quantitative tool for explosive residue analysis.

*Keywords:* Hydrogel; Digital images; Red Green Blue colour system; iPhone; Trinitrotoluene

## 1. Introduction

Trinitrotoluene (TNT) is a widely used explosive material. It is known to have contaminated soil and water as a consequence of its use in military activities, the operational deployment of land mines, and in other industrial activities [1-3] which has led to significant environmental pollution. A wide range of instrumental methods have been applied for the detection of TNT such as ion mobility spectroscopy, capillary electrophoresis, and gas or liquid chromatography [3-12]. Although the instruments used to conduct such tests are highly efficient, they are expensive and are not available in many laboratories. Field detection of TNT using various sensors has also been reported [13, 14]. Although colourimetric sensors based on the entrapment of specific reagents within solid supports provides a low cost, portable, and flexible means for the rapid on-site qualitative detection of TNT, a spectrophotometer is required for quantitative analysis [1, 15, 16]. In this article, a low cost colourimetric test kit was developed based on the synthesis of hydrogel material and used incorporately with digital image colorimetry for quantitative analysis.

Poly vinyl alcohol (PVA) has been widely used in the manufacture of adhesives and emulsifiers as well as in the textile and paper industry [17]. This synthetic water soluble hydrophilic polymer is commonly used to prepare new materials because it is non-toxic, non-carcinogenic, biodegradable and biocompatible. It can also be used to form gels with tailored morphologies, blended with other polymers, or to form the matrix for different fillers [18].

A three-dimensional cross-linked PVA network, PVA hydrogel, can absorb and retain large amounts of water and biological fluids [19]. It can be used to prepare a stimulus-sensitive material, capable of detecting, for example, pH, temperature, pressure, stress, ionic strength, electric field and light [20, 21]. Such gels have been used in various applications including tissue engineering, drug delivery systems or biomedical engineering. It has been applied as a sensor by using a transducer to convert the swelling of the hydrogel to an optical or electrical output [21, 22] and used for pollutant sorption of dyes [18] and uranyl ions [23] by being blended with other materials.

PVA hydrogels can be synthesized by either a chemical or physical cross-linking process [24]. Chemical cross-linking can be conducted by irradiation or the use of bifunctional cross-linkers such as glutaraldehyde, while physical cross-linking can be achieved by the use of freezing/thawing cycles to form crystals. The hydrogel made by physical cross-linking processes produce a gel with a larger mesh or pore size [25] but with poorer mechanical properties and less thermal stability than gels formed by chemical cross-linking [26]. The properties of the synthesized material can be improved by the combination of cryogenic treatment with a chemical cross-linking process which can induce both physical and chemical cross-linking [27]. The combination of the two methods of synthesis produces an elastic and sponge-like cryogel with good mechanical stability and high flow-through pores [28, 29].

This article reports the successful synthesis of a PVA hydrogel as a low cost colourimetric test kit for the simple and rapid TNT detection for the first time. The PVA hydrogel test kit was formed using a combination of a chemical cross-linking process and cryogenic treatment. Ethylene glycol diglycidyl ether (EGDE) was used as the chemical cross-linker, while potassium hydroxide (KOH) was used as the colourimetric reagent. Entrapped KOH was released from the hydrogel matrix to react with TNT producing a specific colour product. Quantitative analysis was obtained through digital image colourimetry using an iPhone and delivered reliable and validated results removing the necessity for spectrophotometric detection.

## **2. Materials and methods**

### *2.1. Materials*

2,4,6-Trinitrotoluene in acetonitrile ( $1 \text{ mgmL}^{-1}$ ) was purchased from Supelco (Bellefonte, PA). Potassium hydroxide (KOH) was produced by Carlo Erba (Spain), and acetone was purchased from Merck (Darmstadt, Germany). Poly vinyl alcohol ( $M_w$  85000-124000  $\text{gmol}^{-1}$ , >99% hydrolyzed) and poly ethylene glycol diglycidyl ether (EGDE, 98%) were purchased from Sigma-Aldrich Ltd. Ultrapure water was obtained from a Barnstead EasyPure II laboratory water purification system (Barnstead EasyPure II, Thermo fisher scientific, OH).

## 2.2. *Preparation of the PVA hydrogel TNT test kits*

The PVA hydrogel TNT test kits were prepared by modifying the procedure reported in previous work [23, 28, 29]. A PVA solution was prepared by dissolving PVA granules in ultrapure water at 100°C and stirred for 30 minutes to obtain a clear viscous solution. It was cooled at room temperature before the addition of the colourimetric reagent and the cross-linker. The colourimetric reagent, KOH solution was added to the PVA solution and stirred for two minutes. Finally, EGDE solution was added as a cross-linker and the solution stirred for a further minute. The polymer mixture was then transferred into micro-PCR tubes and kept in a freezer overnight prior to further use. Blank hydrogel test kits were also prepared using the same procedure without the addition of KOH.

For optimization of the test kit, various of combinations of the necessary ingredients including concentrations (2.5, 5.0, 7.5, 10% w/v) and volumes (0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0mL) of PVA solution, concentrations (0.05, 0.1, 0.25, 0.5, 1.0 molL<sup>-1</sup>) and volumes (0.25, 0.5, 1.0, 1.5, 2.0mL) of KOH solution, volumes of EGDE (25, 50, 75, 100µL), and volumes of polymer mixture (25, 50, 75, 100µL) were studied. The optimum hydrogel recipe was chosen as that which produced the darkest color product of TNT using the least amount of chemicals.

## 2.3. *Characterization of the PVA hydrogel TNT test kits*

The morphology and functional groups of the PVA hydrogel TNT test kits were investigated using a scanning electron microscope (SEM-Quanta400, FEI, Czech Republic) and Fourier transform infrared spectroscope (FTIR-Equinox55, Bruker, Germany). Spectral analysis of the FTIR was performed over a range of 4000 to 400 cm<sup>-1</sup> at a resolution of 4 cm<sup>-1</sup> using KBr pellet preparation.

## 2.4. *Colour test of TNT*

A series of TNT standard solutions in the range of 0.5 to 100 mgL<sup>-1</sup> were prepared by diluting a stock solution (1 mgmL<sup>-1</sup> in acetonitrile) with acetone. Each TNT standard solution was transferred into five PVA hydrogel TNT test kits (100µL each) and mixed for one minute. The red-brown products obtained were then photographed using the built-in digital camera of an iPhone 4.0, six

images of each test kit being taken. The average intensities of the red, green and blue colors (RGB values) across the six images from the five test kits at each concentration were studied using an in house RGB analysis program.

#### 2.5. *Photographic system for quantitative analysis of TNT*

A photographic box (13.5cm×15.5cm×7.5cm) made of opaque black corrugated plastic board with an internal white background and 24 high intensity white light-emitting diodes (LEDs) was used throughout this work. Five PVA hydrogel TNT test kits were hung at the top of the box and sealed with a small cover to eliminate any effects from environmental light. The red-brown products obtained from the five test kits were captured through an aperture in the side of the box using the built-in digital camera of an iPhone 4.0 which was set with the flash off and automatic white balance on. The high dynamic range (HDR) mode was switched off and the images were recorded as original 1.05 MB (2592×1936-pixel) JPEG (24-bits) images on the iPhone's memory.

All the images from the iPhone's memory were transferred to a computer before the RGB values were analysed using an in house RGB analysis program. A 30×30 pixel patch of the image from each test kit was initially manually selected from one of the six recorded images for colour analysis. Equivalent image patches from the other five digital images for each test kit were then automatically selected by the image analysis program. The calibration curve for quantitative analysis was established by using the average RGB values from all six images from the five test kits (30 values in total) as a single data point for each standard concentration.

#### 2.6. *Colour test of TNT in soil sample*

Four post-blast soil samples were prepared using the same procedure as in previous studies [30-32]. Briefly, a 1 gram soil sample was extracted using 5mL of acetone before being sonicated for 10 minutes and filtered using Whatman No.1. The four post-blast soil extracts were then analysed using the PVA hydrogel TNT test kits. A blank soil sample was prepared using the same procedure. A TNT standard solution (5 mgL<sup>-1</sup>) was spiked into the blank soil sample before and after extraction to

investigate the efficiency of the extraction method (%recovery) and the effect of the colour of the soil filtrate on the colour product.

### 2.7. System performance and method validation

The analytical performance of the test kit and the digital image colourimetry, *e.g.* linear range, calibration equation, linearity, sensitivity, precision, and accuracy were investigated. Precision in terms of percentage relative standard deviation (%RSD), both intra- and inter-day for each color component, for six replicates of six images was investigated over a period of three days. The accuracy was investigated in terms of the percentage relative accuracy (%RA). This was achieved by analysing a sample of a known concentration of TNT ( $x_{\text{control}}=7.5 \text{ mgL}^{-1}$ ) and that concentration was then quantified using the established calibration curve ( $x_e$ ). The limit of detection (LOD) was calculated based on the standard equation,  $y_{\text{LOD}}=y_B+3S_B$ , where  $y_{\text{LOD}}$  is the  $y$  value at the limit of detection,  $y_B$  is the  $y$ -axis intercept, and  $S_B$  is the standard deviation of the slope of the calibration graph [33].

## 3. Results and discussion

### 3.1. Preparation and characterization

The PVA hydrogel TNT test kits were synthesized by a combination of cryogenic treatment and chemical cross-linking using EGDE as a bifunctional agent instead of glutaraldehyde. Although glutaraldehyde is a common bifunctional agent [29, 34], its toxicity and the non-uniformity of the obtained matrix [19] were matters of concern. The optimization studies found that 1mL of 5% w/v PVA solution, 1mL of  $0.25 \text{ molL}^{-1}$  KOH as a colourimetric reagent, and 25 $\mu\text{L}$  of EGDE provided an elastic hydrogel (Fig. 1) which produced the darkest colour product with TNT.

#### **Insert figure 1**

The SEM images of the PVA hydrogel TNT test kit (Fig. 2a) revealed a homogenous entrapment of KOH molecules within the hydrogel matrix. The presence of this colourimetric reagent within the hydrogel matrix was confirmed by the presence of the large absorption bands at  $1356 \text{ cm}^{-1}$  and  $1442 \text{ cm}^{-1}$  [35] in the FTIR spectrum of the material (Fig. 2b). These entrapped KOH molecules

will be released after an addition of sample solution. This made the reaction between TNT and colourimetric reagent occurred and finally produced the colour product. A band with maximum absorption at  $3266\text{ cm}^{-1}$  was attributed to the O-H stretching vibration peak from the PVA [17, 36, 37], while the maximum absorption peak at  $1096\text{ cm}^{-1}$  can be attributed to C-O-C stretching vibration from the cross-link reaction of the PVA and the EGDE [23, 38]. The stretching vibration of the alkyl groups ( $\text{R-CH}_2$ ) from the EGDE [38] and/or the PVA [26] was observed at a wave peak between 3000 and  $2850\text{ cm}^{-1}$  with the maximum peak at  $2910\text{ cm}^{-1}$ .

### **Insert figure 2**

The reproducibility of the TNT quantification in terms of %RSD for 20 test kits prepared at the same time was in the range of 0.09-1.22%RSD for the measured RGB intensities. When 20 test kits were prepared for 4 times (5 test kits each), the %RSDs obtained were in the range of 0.39-1.35%.

### *3.2. Colourimetric test of TNT*

A colourimetric test based on the formation of Janowsky complexes from the nitro-aromatic rings of TNT with alkaline acetone [15, 16, 39, 40] was conducted using potassium hydroxide, KOH, as a strong inorganic alkaline [40-43] (Fig. 3a). The addition of a clear TNT standard solution into the test kit container immediately produced a red-brown product (Fig. 3b) different from the red-violet product obtained from the KOH solution without the hydrogel matrix (Fig. 3c). This red-brown product remained for 4 minutes before gradually changing to red, while the red-violet products from the KOH solution gradually changed to red within 15 minutes. The red-violet product from the KOH solution and the red-brown complex from the hydrogel kit thus seem to be an intermediate form of the red Janowsky complex of TNT.

The green and blue channels from the RGB analysis of the digital images of the red-brown products revealed constant intensities for 4 minutes before increasing (Fig. 4a), while the red-violet products obtained from the TNT reaction with the KOH solution without the hydrogel matrix showed



constant intensities for 15 minutes (Fig. 4b). It seems that the hydrogel matrix shortens the period of presence of the red-violet intermediate form of the Janowsky complex.

**Insert figure 3**

**Insert figure 4**

A purple-blue product was observed after the addition of 1,3-dinitrobenzene (1,3-DNB) (Fig. 3d), while a blue product was obtained from standard solutions of 2,4-dinitrotoluene (2,4-DNT) (Fig. 3e) for 6 minutes post reaction. When 4-nitrotoluene (4-NT; 1000 mgL<sup>-1</sup>) was analysed, no colour change was observed. These colour products were easily distinguished from the red-brown product of the TNT complex. The colour of all the complexes at 2.5 mgL<sup>-1</sup> could be observed by eye indicating the high sensitivity of the test kit for these compounds. The highest sensitivity, in the range of 1-10 mgL<sup>-1</sup>, was obtained from the 1,3-DNB complex with those from the 2,4-DNT and TNT being lower (8.9±0.4 (I<sub>G</sub>), 8.8±0.3 (I<sub>R</sub>) and 6.7±0.4 a.u.Lmg<sup>-1</sup> (I<sub>B</sub>), respectively). When the mixture of TNT, DNB, and DNT was tested, the red-brown product of TNT immediately occurred before slowly changing to violet-blue as DNB and DNT product. The product is finally stable as the mix color of red-brown, purple, and blue which is different from each individual product.

### 3.3. *Digital image colourimetry for the quantification of TNT*

The quantification of TNT using the PVA hydrogel TNT test kit was achieved by using digital image colourimetry (DIC). Digital images of the red-brown complexes produced by TNT were captured as previously detailed. The intensity of the red light (I<sub>R</sub>) was lower than the green and blue light at low concentrations of TNT (< 1 mgL<sup>-1</sup>), but was the highest at high concentrations (Fig. 5a) because of the presence of darker red-brown products (Fig. 3b).

**Insert figure 5**

The calculated molecular absorption [44-46] of the red-brown products was also investigated. The blue channel (400-500nm) revealed the highest level of absorbance (Fig. 5b) corresponding to the reported maximum absorption wavelength ( $\lambda_{\max}$ ) at 462nm using a spectrophotometer [40]. The

calculated absorbance of the red-brown complex increased with TNT concentrations from 0.5-40 mgL<sup>-1</sup> before becoming constant. The total intensity ( $I_{\text{Total}}$ ) (Fig. 5c) and the total absorbance ( $A_{\text{Total}}$ ) (Fig. 5d) were also investigated and revealed similar results to other measurements reported.

#### 3.4. System performance and method validation

The calibration equations for the quantification of TNT using the PVA hydrogel TNT test kit and DIC are summarized in table 1, while the analytical performance is shown in table 2. The red channel provided the lowest sensitivity for both intensity and calculated absorbance. The highest sensitivity was achieved from the total intensity ( $I_{\text{Total}}$ :  $12.1 \pm 0.8$  a.u. Lmg<sup>-1</sup>), while the widest linear range was obtained from the intensity of the red channel (0.5-100 mgL<sup>-1</sup>). This was wider than previously reported using a spectrophotometer (5-50 mgL<sup>-1</sup>) [1]. The limit of detection ( $0.32 \pm 0.01$ mgL<sup>-1</sup>) was obtained from the calculated absorption of green light and was better than those recently reported using colourimetry coupled with spectrophotometric detection (3 mgL<sup>-1</sup>) [1]. Precision was in the range of 0.42-1.60%RSD for intra-day analysis and 0.12-3.60%RSD for inter-day (over three days). Good accuracies (93-108%RA) were obtained for almost all measurements, except the calculated absorbance of red light (130%RA).

**Insert table 1**

**Insert table 2**

#### 3.5. Stability

The PVA hydrogel TNT test kits (n=225) were prepared using the same ingredients at the same time in order to evaluate their stability. Three test kits were tested with TNT on the day of preparation, while the remaining kits (n=222) were split into three groups and stored in a freezer at -18°C, and outside and inside a desiccator at 29°C. Three kits were removed from each group for TNT detection every two days over a one-month period and then on a weekly basis for 3 further months. The intensities of the red, green and blue channels from the test kits stored in the freezer had altered by +6.3%, +5.1%, and -4.9% respectively after 3 months, while the RGB values from the test kits

stored inside the desiccator had reduced by 7.8%, 30%, and 25% after a month (29 days). The test kits stored outside the desiccator produced the worst stability with the RGB values reducing by 12%, 22%, and 24% after 27 days. On the basis of these results, storage of the test kits in a freezer is recommended.

### 3.6. *Reusability*

The reusability of the PVA hydrogel TNT test kit was investigated by applying a new test after washing the test kit with acetone. It was found that the RGB values taken from the red-brown product changed by -5.4%, +0.3%, and +4.0% after 12 uses of the same test kit (Fig. 6). Because one PVA hydrogel TNT test kit costs only 0.5 THB (0.01 USD) to produce, the fact that each test can be used 12 times indicates that one test actually costs less than 0.001 USD.

**Insert figure 6**

### 3.7. *Quantification of TNT in soil*

A quantity of 5 mgL<sup>-1</sup> standard TNT solution was spiked into six blank soil samples before and after extraction to investigate the efficiency of the extraction method and the influence of soil matrices. A percentage recovery of 103.6±0.8% and %relative accuracy of 98.33±0.02% were obtained. These results indicate good extraction efficiency and detected no influence from the soil colour.

The test kits were applied to four post blast samples recovered from soil. Two of these provided positive results with the kits (S1 and S2 in Fig. 7d) with concentrations of 0.011 and 0.06 mg g<sup>-1</sup>. The results were in good agreement with spectrophotometric data obtained from the same samples (0.012 and 0.06 mg g<sup>-1</sup>) indicating excellent performance of the hydrogel TNT test kits when combined with DIC for these types of samples.

**Insert figure 7**

### 3.8. *Application on Bangkok bombing case (Ratchaprasong intersection) proof of concept*

The hydrogel TNT test kit was deployed to detect trace explosive residues at the scene of the Ratchaprasong bomb explosion a week after the bombing. Positive results for TNT were obtained from the scene as shown in Fig. 7a-c. The results were in good agreement with other in-house developed test kits [32].

## 4. Conclusion

A cost effective PVA hydrogel TNT test kit was successfully developed for trinitrotoluene detection. The test kit was easy to transport and use as it was fabricated within a small flat cap micro-PCR tube where all that is required is the addition of a small volume of the sample solution into the test kit tube during testing. The PVA hydrogel TNT test kit was stable for 3 months when kept in a freezer and was able to be reused 12 times without significant loss of accuracy. Rapid quantitative and accurate analysis of trinitrotoluene was achieved by using the test kit in combination with DIC.

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## Figure captions

**Fig.1** The PVA hydrogel TNT test kit.

**Fig. 2** (a) Scanning electron microscopic (SEM) image (b) FTIR spectrum of the PVA hydrogel TNT test kit.

**Fig.3** (a) Chemical reaction of TNT and KOH solution [15-16] (b-e) Colourimetric products of (b) TNT (d) 1,3-DNB (e) 2,4-DNT using the PVA hydrogel TNT test kit, and (c) TNT using KOH solution without hydrogel matrix.

**Fig. 4** Stability of colourimetric products of TNT (a) using the PVA hydrogel TNT test kit (b) using KOH solution without hydrogel matrix.

**Fig. 5** Relationships of TNT concentrations and (a) RGB intensities (b) the calculated absorbance (c) total intensity (d) total absorbance

**Fig. 6** Reusability of the PVA hydrogel TNT test kit.

**Fig.7** Colourimetric products of (a-c) post blast samples from the scene of Bangkok bombing (at Ratchaprasong intersection) tested using the PVA hydrogel TNT test kit (d) post blast soil samples

**Table 1** Calibration equations for quantification of TNT using the hydrogel TNT test kit and DIC

| <b>Relationships</b>     | <b>Calibration equation</b>                  | <b>Linear rang (mg L<sup>-1</sup>)</b> | <b>R<sup>2</sup></b> |
|--------------------------|--|--|----------------------|
| I <sub>R</sub> and C     | $y = -(0.74 \pm 0.02)x + (114 \pm 1)$        | 0.5-100                                | 0.9928               |
| I <sub>G</sub> and C     | $y = -(4.1 \pm 0.3)x + (121 \pm 3)$          | 2.5-20                                 | 0.9975               |
| I <sub>B</sub> and C     | $y = -(6.7 \pm 0.4)x + (117 \pm 3)$          | 1-10                                   | 0.9972               |
| A <sub>R</sub> and C     | $y = (0.0046 \pm 0.0002)x + (0.32 \pm 0.01)$ | 5-100                                  | 0.9911               |
| A <sub>G</sub> and C     | $y = (0.024 \pm 0.003)x + (0.3 \pm 0.1)$     | 0.5-40                                 | 0.9975               |
| A <sub>B</sub> and C     | $y = (0.0426 \pm 0.001)x + (0.30 \pm 0.02)$  | 0.5-40                                 | 0.9972               |
| I <sub>Total</sub> and C | $y = -(12.1 \pm 0.8)x + (356 \pm 4)$         | 1-10                                   | 0.9916               |
| A <sub>Total</sub> and C | $y = (0.07 \pm 0.03)x + (0.9 \pm 0.6)$       | 0.5-40                                 | 0.9979               |

**Table 2** Analytical performance for TNT detection using the hydrogel TNT test kit and DIC

| Relationships            | Sensitivity (a.u. Lmg <sup>-1</sup> ) | Limit of detection<br>(mgL <sup>-1</sup> ) | Precision (%RSD) |                | Accuracy<br>(%Relative accuracy) |
|--------------------------|---------------------------------------|--|------------------|----------------|----------------------------------|
|                          |                                       |  | Intra-day, n=6   | Inter-day, n=3 |                                  |
| I <sub>R</sub> and C     | 0.74± 0.02                            | 5.8±0.2                                    | 0.80             | 2.63           | 93                               |
| I <sub>G</sub> and C     | 4.1±0.3                               | 1.2±0.1                                    | 1.60             | 0.13           | 108                              |
| I <sub>B</sub> and C     | 6.7±0.4                               | 1.4±0.1                                    | 0.80             | 1.52           | 104                              |
| A <sub>R</sub> and C     | 0.0046±0.0002                         | 6.8±0.3                                    | 0.98             | 3.60           | 131                              |
| A <sub>G</sub> and C     | 0.024±0.003                           | 0.32±0.01                                  | 1.17             | 0.12           | 99                               |
| A <sub>B</sub> and C     | 0.0426±0.001                          | 0.61±0.02                                  | 0.42             | 1.05           | 93                               |
| I <sub>Total</sub> and C | 12.1±0.8                              | 1.3±0.1                                    | 0.99             | 1.55           | 101                              |
| A <sub>Total</sub> and C | 0.07±0.03                             | 2.14±0.04                                  | 0.75             | 1.29           | 93                               |

\* $y_{LOD} = y_B + 3S_B$  [33]