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Rapid detection of nitroaromatic and nitramine explosives on chromatographic paper and their reflectometric sensing on PVC tablets

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ABSTRACT

Rapid and inexpensive sensing of explosive traces in soil and post-blast debris for environmental and criminological purposes with optical sensors has recently gained importance. The developed sensing method for nitro-aromatic and nitramine-based explosives is based on dropping an acetone solution of the analyte to an adsorbent surface, letting the solvent to dry, spraying an analytical reagent to produce a persistent spot, and indirectly measuring its reflectance by means of a miniature spectrometer. This method proved to be useful for on-site determination of nitroaromatics (trinitrotoluene (TNT), 2,4,6-trinitrophenylmethylnitramine (tetryl) and dinitrotoluene (DNT)) and nitramines (1,3,5-trinitro-1,3,5-triazacyclohexane (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)) pre-adsorbed on a poly vinyl chloride (PVC) surface, with the use of different spray reagents for each group of explosives producing different colors. The calibration equations of the tested compounds as reflectance vs. concentration showed excellent linearity (correlation coefficient: 0.998–0.999). The linear quantification interval in terms of absolute quantity of analyte was 0.1–0.5 μg. The developed method was successfully tested for the analysis of military explosives Comp B and Octol, and was validated against high performance liquid chromatography (HPLC). The reflectometric sensing method could also be used for qualitative identification of the nitrated explosives on a chromatographic paper. The reagent-impregnated paper could also serve as sensor, enabling semi-quantitative determinations of TNT and tetryl.

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1. Introduction

Fast and practical on-site analysis of nitroaromatic and nitramine type military explosives, preferably with the use of sensors, is important for preventing terrorist acts, for reclamation/remediation of contaminated land previously used for military purposes (e.g., explosive manufacture or ammunition dumping), and for forensic investigations. While trace explosive vapor detection methods requiring more sophisticated instrumentation continue to be plagued by the low volatility of many target analytes [1], the more reliable HPLC methods suffer from long analysis times at the order of tens of minutes. Mass spectroscopic methods coupled to LC/GC [2] show a great promise in explosive detection, but are still held back from routine portable application by their high cost and size [1]. Differential reflection spectroscopy in the ultraviolet–visible (UV–Vis) region may be used to distinguish between the reflectivities of two adjacent parts of a specimen with and without adsorbed trinitrotoluene (TNT) [3]. Other sophisticated instrumental techniques used in this regard are surface enhanced Raman spectroscopy (SERS), nuclear quadrupole resonance, energy-dispersive X-ray diffraction (XRD), neutron activation analysis (NAA), cyclic voltammmetry (CV), and ion mobility spectrometry (IMS) [4].

Color reactions were also developed for soil contamination screening methods. Our research group has carried out extensive work on spectrophotometric determination of TNT by improving the water tolerance and color stability of the TNT-hydroxide or TNT-acetonate charge-transfer complexes formed as Meisenheimer and Janowsky anions, respectively [5,6]. A commercial colorimetric system using proprietary reagents is available as the 'EXPRAY Field Test Kit', with components appropriate for 'Group A' explosives (nitro-aromatics), 'Group B' explosives (RDX, HMX, pentaerythritol tetranitrate (PETN), etc.), and for inorganic nitrates [7], however such a commercial system neither has freely available reagents nor is capable of quantitative measurement. Electrochemical sensors not properly responding to mixtures may suffer from limited sensitivity and electrode fouling, while immunosensors are good for detecting explosive residues in soil but not for airport security screening applications [8].

Chemical sensors, portable detectors and analysis kits provide an on-site possibility of real-time analysis, exemplified by silicagel-coated [9] and amine-loaded PVC membrane [10] TNT sensors. Our

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research group developed a low-cost, absorption-based colorimetric sensor for TNT assay using dicyclohexylamine (DCHA) as the charge-transfer reagent [11] immobilized on a PVC membrane [12]. Several kinds of colorimetric chemical sensors have been developed for rapid on-site detection of explosives and their impurities and/or degradation products [7,9,10,13–19].

Reflectance spectrometry is a valuable alternative for the analysis of solid samples on a support [20] where spectral composition of surface-reflected optical radiation is analyzed with respect to its angularly dependent intensity and the composition of incident radiation [16]. Direct analysis of solid samples is possible, and the elution step can be completely eliminated from the colorimetric solid-phase extraction (C-SPE) process [21]. Thus, a simple, selective and rapid reflectometric sensing technique was developed in this work for nitro-aromatic and nitramine explosives. The proposed procedure is different from on-site colorimetric sensing applications (like 'EXPRAY Field Test Kit' [7]) which merely give qualitative information by color comparison, because it yields quantitative results and all its reagents are nonproprietary with definitely known composition. Sampling, reagent addition, and reflectance reading (using a miniature spectrophotometer) were made on PVC tablets. The developed method was successfully applied to synthetic and real (e.g., Comp B and Octol) explosive mixtures containing nitramine and nitro-explosives (such as TNT + RDX or TNT + HMX), and statistically validated against HPLC [22].

2. Materials and methods

2.1. Chemicals

The explosive materials; TNT, tetryl, DNT, RDX, HMX, Comp B composite explosive (containing 60% RDX, 39% TNT, and 1% wax), Comp A5 (99% RDX and 1% filler material), Comp C4 (91% RDX, 2% polyisobutylene, 5.3% dioctyl sebacate, and 1.6% oil), Hexal P30 (67.5% RDX, 30% metallic Al, 0.5% graphite) and Octol composite explosive (containing 70% HMX, 30% TNT) were kindly supplied by Makine Kimya Endustrisi Kurumu (MKEK: Machinery & Chemistry Industries Institution) through the supervision of Milli Savunma Bakanligi, Teknik Hizmetler Daire Baskanligi (Ministry of National Defence, Office of Technical Services) of Turkey. All other reagents were from E. Merck (Darmstadt, Germany), Sigma–Aldrich (Deisenhofen, Germany) and Fluka (Neu-Ulm, Germany). The solvent used in preparation and dilution of explosive solutions was HPLC-grade extra pure acetone (E. Merck). The solid support for reflectometric measurements was high molecular weight PVC (Fluka). In semi-quantitative determination of nitro-aromatics and nitramines on paper matrix, 3MM CHR chromatography paper (Cat. No: 3030-861) was used.

2.2. Solutions

The standard stock solutions of individual explosive materials and military-purpose explosive mixtures (Comp B, Octol, Comp A5, Comp C4, and Hexal P30) at 1000 mg L^{-1} concentrations were prepared in extra pure acetone. The working solutions to be tested with the developed rapid colorimetric sensing were prepared from these stock solutions by dilution with pure acetone in the concentration range of 10–40 mg L^{-1} for TNT, 10–50 mg L^{-1} for teryl, DNT, RDX and HMX.

2.3. Reagent A (for nitro-aromatic explosives)

To a 100 mL flask were added 2 g sulfanilamide, 70 mL dimethylsulfoxide (DMSO), and 30 mL of 5% (w/v) KOH solution in 2:3 (v/v) methanol/isopropanol mixture, stirred on a magnetic stirrer for 1 h, and rapidly filtered under vacuum. This solution is significantly influenced by oxygen and light, and therefore was kept after N_2 purging in a dark colored bottle, and used having minimum contact with air [23].

2.4. Reagent B (for nitramine explosives)

To a 100 mL flask were added 0.3 g α-naphthylamine, 10 mL *ortho*-phosphoric acid (85%), and 90 mL bidistilled water, and stirred on a magnetic stirrer. Hydrazine sulfate (0.5 g) and sodium thiosulfate pentahydrate (0.05 g) were added under continued stirring, and finally stirred for 1/2 h more after addition of 0.1 g active carbon. This mixture was rapidly filtered under vacuum through a Whatman blue-band quantitative filter paper. Since this solution is significantly influenced by oxygen and light, it was kept (after N_2 purging) in a dark colored bottle, and used having minimum contact with air [23].

2.5. Instruments

Experiments were carried out using a commercially available miniature fibre-optic based spectrometer (Ocean Optics Inc., HG4000CG-UV-NIR) which utilizes a small tungsten halogen lamp as the light source and a CCD based detector for reflectance measurements. Light reflected from the flow cell was transmitted by a bundle of optical fibres to a miniature fibre-optic spectrophotometer which on the other hand was connected to a PC (Dell-compatible) and also a printer. For optical isolation, the flow cell and the detector were kept in a black box to minimize any interference from ambient light. The spectral deconvolution was performed after smoothing the spectra by a 20-point Fourier transform filter (FTF) using peak fitting module in OriginPro 6.0 software (OriginLab Co., USA).

For comparative assays of RDX, TNT, and HMX with HPLC, a Waters 2695 Separation Module (Waters 2998 PDA (photo diode array) detector) HPLC chromatographic instrument equipped with a MZ Hypersil ODS C_{18} (7 μm), 125 × 4.6 mm ID reversed phase (RP)-column was used in conjunction with a UV (254 nm) detector and a 40% MeOH + 60% H_2O (v/v) mixture mobile phase at a flow rate of 2 mL min^{-1}. The injection volume was 25 μL, and the retention time of the main explosive peaks were 1.317 min for HMX, 2.061 min for RDX, and 4.98 min for TNT.

2.6. Experimental set-up and recommended procedure for the determination of nitro-aromatic and nitramine explosives

The developed procedure is based on dropping the explosive solution on a polymeric solid adsorbent, colored product formation on the adsorbent surface with reagent addition, and measuring its reflectance with the aid of a reflectance spectrometer. Weighings of 0.2 g were taken from humidity-free polyvinyl chloride (PVC), and converted with the aid of a Specac press into 1.2 cm-diameter and 1 mm-thickness tablets under 12 tons of pressure. The processed tablets were further dried (to prevent latent cracks) in a 50 °C oven for 1 h. A volume of 10 μL of acetone solution of the explosive was dropped on the tablet, and let to stand for 5 s for the excess acetone to evaporate. Nitro-aromatic group reagent, reagent A (10 μL) was then dropped on this tablet, and let to stand for 5 min for full color development; the colors formed were purplish brown for TNT, orange for tetryl, and greenish blue for DNT. To test the presence of nitramines, reagent B (10 μL) was then dropped and let to stand for 15 min, and RDX and HMX formed a pink color on the tablets. The spots due to nitramines formed on the previous spots due to nitro-aromatics without interference, enabling the determination of both explosive groups on the same tablet. The reflectometric background was recorded before each measurement, i.e., blank auto-zero (with tablets containing only the reagents) was made before each group reading at identical times with the samples. When the sequence of additions was interchanged (i.e., via sample addition onto immobilized reagents-containing tablets kept in a vacuum dessicator), cracks were visualized on the tablets, causing erratic readings. Detection limits (LOD) were estimated by measuring the standard deviations (σ_{bl}) of near-blank level sample readings, and using the equation: LOD = 3 σ_{bl}/m, where m was the slope of the calibration line drawn as reflectance vs. concentration, whereas quantitation limits (LOQ) were accepted as (10/3) LOD.

2.7. Investigation of the effects of possible interferents (nitrite, nitrate, weak acids, and humates)

All tests were carried out in 90% (v/v) acetone solution in water for solubilizing the nitrite constituent and to simulate wet soil taken up with acetone so as to contain nitramine explosive residues in the extract. In the analysis of 20 mg L^{-1} RDX or HMX with the proposed method, $NaNO_2$ was added at varying concentrations up to 10-fold (w/w), and either $NaNO_3$ or NH_4NO_3 up to 50-fold (w/w) ratios. For simulating the conditions for dried soil (assumed to be contaminated with nitramines), anhydrous acetone solutions of $NaNO_2$ (i.e. saturated solution) were used.

The dependence of the analytical results for nitro-aromatics on the initial pH of the solution was tested with a number of weak acids and humates that may also be found in soil and groundwater. The tests were carried out by adding 0.5 mL H_2O and 35 μL aqueous weak acid solution (or humate) at varying concentration to 5 mL of a 50 mg L^{-1} TNT solution in pure acetone, and the concentrations of the tested acids as well as the initial pH values were recorded. All interferent-containing samples were reflectometrically analyzed on PVC tablets, as described in the standard procedure.

2.8. Validation of the proposed assay against HPLC

The proposed method was compared with HPLC for validation, as described in literature [22]. Briefly, a concentration range of 5–100 mg L^{-1} for TNT, RDX and HMX standard solutions in acetone was assayed by HPLC to construct the calibration curves by recording the peak areas vs. concentration. As real sample materials, 25.0 mg samples each (N = 5) of Comp B composite explosive (containing 39% TNT and 60% RDX) and Octol composite explosive (containing 30% TNT and 70% HMX) were separately dissolved in 25 mL acetone, 1.25 mL of these stock solutions were diluted with acetone to a final volume of 25 mL to yield 50 mg L^{-1} Comp B and 50 mg L^{-1} Octol solutions to be assayed with HPLC. Statistical comparisons between the findings of the recommended (reflectometric) and reference (HPLC) methods were made with the aid of t- and F-tests.

2.9. Study of synthetic and real mixtures

Synthetic binary mixture solutions containing RDX + TNT and TNT + HMX (in various mass ratios) were prepared by mixing 1000 mg L^{-1} stock solutions and diluting with acetone to final concentrations of 10–40 mg L^{-1} of each constituent. Likewise, appropriate dilutions were made from 1000 mg L^{-1} acetone solutions of real mixtures (composite materials) to yield final concentrations of 30 mg L^{-1} of Comp A5 and Comp C4, and 50 mg L^{-1} of Hexal P30. Both synthetic and real mixtures were finally analyzed by the "Recommended procedure for the determination of nitro-aromatic and nitramine explosives". The analytical wavelengths of maximum reflection for the tested explosives were as follows: nitroaromatics: TNT: 657 nm, tetryl: 680 nm, and DNT: 639 nm with reagent A; nitramines: RDX: 569 nm and HMX: 548 nm with reagent A + reagent B.

2.10. Semi-quantitative determinations with chromatographic paper

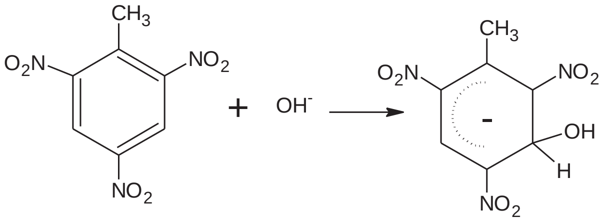
3MM chromatographic paper was tested for the possibility of being used as an explosive sensor by changing the application sequence for tablets. In other words, the reagent (10 μL) was first dropped on the paper, let to dry for 5 min, then the analyte solution (10 μL) added, and let to dry for 10 more minutes. Each analysis was ended with a reflectometric measurement on the paper. The reagent-impregnated chromatographic paper specimens were also kept for 24 h in a vacuum dessicator in the dark before being tested as possible sensors. For testing the speed and stability of color formation, successive application of reagent A and TNT sample solution was made on the chromatographic paper; the reflectance spectra of the produced spot were recorded every minute in the time interval of 1–10 min. For testing the presence of nitramines (i.e., RDX and HMX) on the same paper, reagent A and reagent B were successively added, and the spotted paper was dried with a turbo hair dryer for 1 min; the reflectance spectra of the stabilized spot were recorded every minute starting from the second up to the tenth minute. Samples containing both TNT and nitramines (such as Comp B or Octol) were subjected to the same procedure using both reagents A & B.

2.11. Statistical analysis

Descriptive statistical analyses were performed using Excel software (Microsoft Office 2003) for calculating the means and standard error of the mean. Results were expressed as the mean ± standard deviation (SD). Method validation against HPLC determination of explosives was made by means of Student (t-) and F-tests for the statistical comparison of population means and variances, respectively.

3. Results and discussion

The developed reflectometric methods are based on Meisenheimer anion [24] formation from nitro-aromatics using reagent A (Eq. (1)) and diazo-coupling product formation from nitramines with Griess reaction [25] using reagent B (Eq. (2)):



(1)

The presence of sulfanilamide in reagent A has a double-fold aim: this compound enhances the formation of colored charge transfer (CT)-complexes (Eq. (1)) from nitro-aromatics (i.e., colored Meisenheimer anions of nitro-aromatics can simply form with the alkaline constituent of reagent A), and additionally, it participates in diazonium salt formation in Eq. (2). Chemical reactions involved in the measurement of NO_2^- using the Griess reagent [25] are summarized in Eq. (2). According to the Griess reaction sequence, RDX and HMX are partly reduced to nitrite with hydrazine and thiosulfate constituents of reagent B in alkaline medium, which subsequently forms a diazonium salt with sulfanilamide and cou-

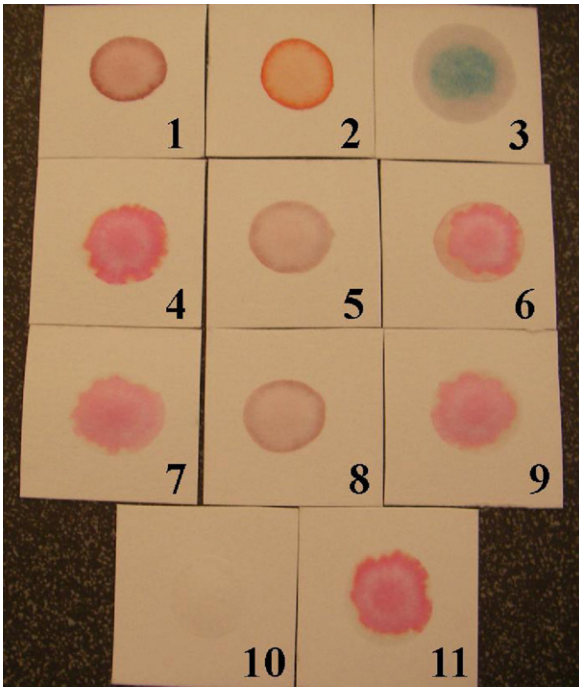
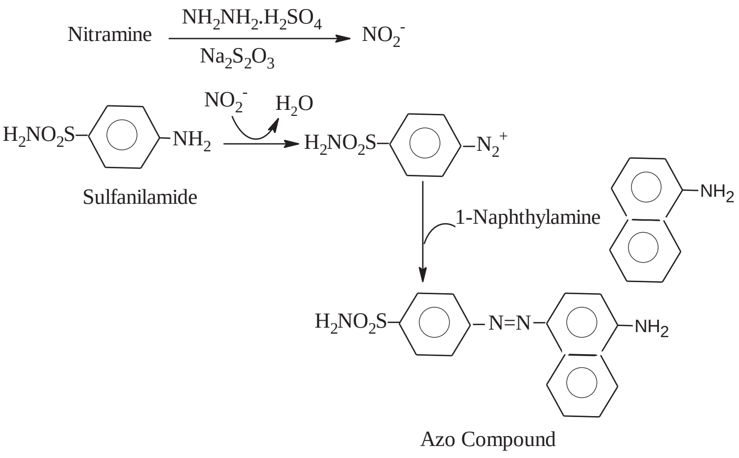


Fig. 1. Colored spots formed on chromatographic paper from nitro-aromatics and nitramines with reagent A and reagent B, respectively. Spots 1, 2, 3, 5, 8 and 10 were formed with reagent A, and spots 4, 6, 7, 9 and 11 with reagent B (successively applied after reagent A). The analytes were; 1: 50 mg L^{-1} TNT, 2: 50 mg L^{-1} Tetryl, 3: 200 mg L^{-1}DNT, 4: 50 mg L^{-1} RDX, 5: 20 mg L^{-1} TNT constituent of Comp B, 6: 30 mg L^{-1}RDX constituent of Comp B, 7: 50 mg L^{-1} HMX, 8: 15 mg L^{-1} TNT constituent of Octol, 9: 35 mg L^{-1} HMX constituent of Octol, 10: No characteristic chromophore formed from Comp C4 with reagent A, 11: 45 mg L^{-1} RDX constituent of Comp C4 formed with reagent B.

ples to 1-naphthylamine to form an azo-dye (i.e., the chromophore characteristic of nitramines).



(2)

The reflectance spectral baseline was previously adjusted to 100 arbitrary units in the detector, which corresponded to the reflectance of a PVC blank tablet containing reagent A alone (for nitro-aromatics detection) or reagent A + reagent B together (for nitramines that may coexist with nitro-aromatics).

The colored spots formed on the chromatographic paper with treatment of nitro-aromatics and nitramines with their characteristic reagents are shown in Fig. 1.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

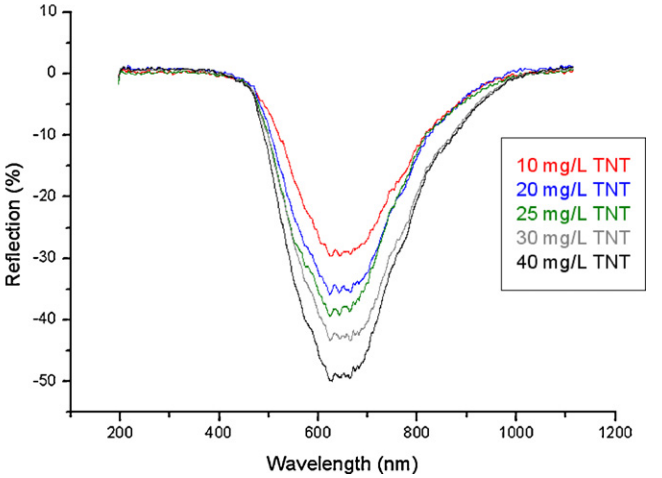


Fig. 2. The reflectance spectra of the chromophore formed on PVC tablet from TNT with reagent A; TNT concentrations: 10–40 mg L^{-1}; spectra were drawn using Origin 6.0 graphics programme and were smoothed using FFT filter with 20 points averaged.

3.1. Quantitative reflectometric determination of nitrated explosives on PVC tablets

The reflectance spectra of the chromophore formed on PVC tablets from TNT with reagent A (Fig. 2), and from RDX with (reagent A + reagent B) combination (Fig. 3) are given as reflectance vs. wavelength for different analyte concentrations.

The absolute amounts of substance being determined in the linear range were between 0.1 and 0.4 μg for TNT, and between 0.1 and 0.5 μg for tetryl, DNT, RDX, and HMX. The fact that the spectral maxima due to nitro-aromatics and nitramines appeared at close wavelengths did not give rise to any interference in analysis, because once reagent B was applied on the chromatographic paper (on which reagent A was applied before), the previous spots due to nitro-aromatics disappeared, and simultaneously, the new spots due to nitramines formed. The slope (m)-intercept (n) values of the calibration lines ($A = mC + n$) as well as the analytical figures of merit, i.e., LOD and LOQ, of the reflectometric sensing method for the tested explosives are shown in Table 1. It was experimentally confirmed that roughly 2 mg L^{-1} TNT, 3 mg L^{-1} tetryl, 5 mg L^{-1} DNT and RDX, and 7.5 mg L^{-1} HMX could be reliably quantified with the proposed method. Three readings were made for each concentration, and the relative standard deviation (RSD, %) of a given set of readings varied in the range 1.0–3.8% for TNT, 1.1–2.6% for

Table 1
Slope-intercept values of the calibration lines and analytical figures of merit for nitro-aromatic and nitramine explosives measured by reflectometric sensing on PVC tablets (second values in parentheses refer to absolute amount of substance determined in μg-units).

	TNT	Tetryl	DNT	RDX	HMX
Slope (*m*)[a]	0.0059 (0.589)	0.0031 (0.306)	0.0093 (0.932)	0.0096 (0.964)	0.0048 (0.482)
Intercept (*n*)	0.233	0.134	0.0305	0.0091	0.0624
Corr. coefficient (*r*)	0.998	0.9991	0.9993	0.9998	0.998
LOD[b] (in initial soln.)	0.64	0.82	1.48	1.45	2.29
LOQ[b] (in initial soln.)	2.13	2.73	4.93	4.83	7.63

[a] In mg^{-1} L units.
[b] In mg L^{-1} units.

tetryl, 0.2–1.6% for DNT, 0.3–3.0% for RDX, and 1.1–3.2% for HMX, depending on the concentration.

3.2. *Effect and treatment of possible interferents*

In the analysis of 20 mg L^{-1} RDX, the relative (positive) errors corresponding to a given excess (w/w) of nitrite were: 3.4% for 5-fold excess, 4.5% for 6-fold, 9.4% for 7.5-fold, and 13.4% for 10-fold excess of NO_2^-. It should be added that, the other major nitramine compound giving a similar reaction, HMX at 20 mg L^{-1} level, was affected from 120 mg L^{-1} NO_2^- with 4.1% positive error. Thus, nitrite level in the determination of nitramines (RDX or HMX) with the recommended procedure should not exceed 120 mg L^{-1} so as to remain within 5% relative error.

On the other hand, under conditions where the sample soil hypothetically contaminated with nitramine explosives is completely dried in an oven and the pulverized sample is taken up with pure acetone, $NaNO_2$ would be basically insoluble in such an acetone solution (i.e. having a solubility less than 1 mg L^{-1}), and in case when such a solution is saturated with sodium nitrite, the resulting nitramine-containing PVC tablets after the application of characteristic nitramine reagent (reagent B) would not have any color contribution from nitrite (i.e. causing less than 0.1% error).

Since nitrate does not give rise to a Griess reaction unless reduced to nitrite, the positive error to RDX determination at 50-fold NO_3^- level was only 0.7%. As ammonium nitrate is usually mixed with nitramine explosives to increase the blast effect, the relative error at 50-fold NH_4NO_3 was also measured, and found to be +0.7%. The relative error to the assay of HMX (20 mg L^{-1}) in the presence of 1000 mg L^{-1} NO_3^- was +3.1%. In short, nitrate was not a serious interferent.

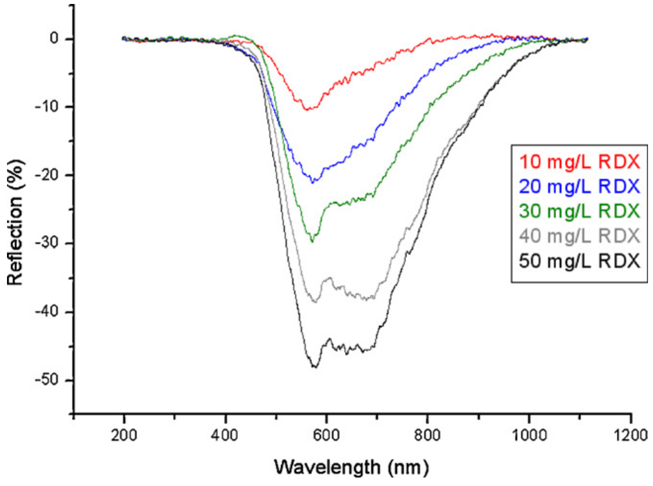


Fig. 3. The reflectance spectra of the chromophore formed on PVC tablet from RDX with reagent A + reagent B; RDX concentrations: 10–50 mg L^{-1}; spectral drawing and smoothing as in Fig. 2.

As for the effect of initial pH of solution on the analysis of nitroaromatics with the proposed method, the relative errors to the measurement of 50 mg L^{-1} TNT caused by weak acids and humate were: −2.16% (0.1 M citric acid, pH = 3.5); +1.4% (0.1 M *ortho*-phosphoric acid, pH = 4.2); −0.85% (saturated ≈0.05 M carbonic acid, pH = 5.1); +0.2% (1 M acetic acid, pH = 5.0); +4.8% (0.2% (w/v) humic acid solubilized with NaOH, pH = 10.0). In the case of humic acid, strong bases should be added to prepare a stock solution, which is dissimilar to actual conditions in soil where humic acids would be in the solid state. In the strong alkaline solution of synthetic soil humates prepared as potential interferents, it is obvious that TNT would immediately form colored Meisenheimer anions without the addition of its characteristic reagent (reagent A) and thus yield positive error, but these conditions would not prevail under ‘field conditions’ in either soil or groundwater. It can be concluded from these experiments that since the nitroaromatics reagent (reagent A) is strongly alkaline, the initial pH caused by weak acids or humates is not so important as the final pH following the addition of reagent would be distinctly alkaline.

3.3. *Method validation*

The proposed reflectometric determination was validated against HPLC [22] on five samples each of Comp B (declared to contain 39% TNT and 60% RDX) and Octol (declared to contain 30% TNT and 70% HMX) each diluted to a final concentration of 50 mg L^{-1} (Table 2). The statistical comparisons of the proposed method with HPLC were performed in discrete solutions containing either RDX or HMX (Table 2) but not both, since neither Comp B nor Octol mixed-explosive formulations contain both nitramines. However, the chromatographic resolution for the HMX and RDX peaks were sufficient for their simultaneous quantitative determination using the reference method [22]. The calibration lines for HPLC determination of pure TNT, RDX, and HMX in the concentration range of 5–100 mg L^{-1} were as follows:

$$\text{Peak Area} = 4.51 \times 10^4 C_{\text{TNT(mg L}^{-1})} - 5.81 \times 10^4 \, (r = 0.9994)$$

$$\text{Peak Area} = 2.48 \times 10^4 \, C_{\text{RDX(mg L}^{-1})} - 3.66 \times 10^4 \, (r = 0.9995)$$

$$\text{Peak Area} = 1.37 \times 10^4 C_{\text{HMX(mg L}^{-1})} - 5.45 \times 10^4 \, (r = 0.9994)$$

Statistical comparisons were made on paired data (Student’s test was a ‘two-tailed’ *t*-test) produced with the proposed and reference methods (sample size of each population: *N* = 5). The applied test was an independent two-sample *t*-test, of equal sample size and equal variance. This test is used when both the two sample sizes (i.e. the number of samples within each group) are equal and it can be assumed that the two distributions have the same variance [26]. The ‘null hypothesis’ was tested at different confidence levels (*t*-test), essentially showing no significant difference between the means of results (Table 2). The precisions analyzed with the *F*-test were alike at 95% confidence level (for all pairs of data, Table 2). The reason for using different confidence levels (only for the *t*-test)

Table 2
Statistical comparison of the proposed sensing method with HPLC for the determination of TNT, RDX and HMX constituents of Comp B and Octol samples.

Sample/analyte	Method	Mean concn.	Std. dev. (σ)	$S^{a,b}$	$t^{a,b}$	$t_{table}{}^{b}$	F^{b}	$F_{table}{}^{b}$
Comp B/TNT	Proposed method	39.47%	0.57%	–	–	–	–	–
Comp B/TNT	HPLC	39.21%	0.71%	0.64	0.64	2.306	0.64	6.39
Comp B/RDX	Proposed method	60.68%	1.09%	–	–	–	–	–
Comp B/RDX	HPLC	62.38%	1.15%	1.12	2.40	2.51	0.90	6.39
Octol/TNT	Proposed method	29.77%	0.69%	–	–	–	–	–
Octol/TNT	HPLC	30.44%	0.53%	0.61	1.74	2.306	1.69	6.39
Octol/HMX	Proposed method	70.60%	0.71%	–	–	–	–	–
Octol/HMX	HPLC	68.59%	1.10%	0.93	3.30	3.36	0.42	6.39

[a] $S^2 = \{(n_1 - 1)\, s_1{}^2 + (n_2 - 1)\, s_2{}^2\}/(n_1 + n_2 - 2)$ and $t_{exp} = (\bar{a}_1 - \bar{a}_2)/\{S(1/n_1 + 1/n_2)^{1/2}\}$ where S is the pooled standard deviation, s_1 and s_2 are the standard deviations of the two populations with sample sizes of n_1 and n_2, and sample means of $\bar{a}_1$ and $\bar{a}_2$, respectively (t has $(n_1 + n_2 - 2)$ degrees of freedom); here, $n_1 = n_2 = 5$.

[b] Statistical comparison made on paired data produced with the proposed and reference methods; the results given only on the row of the reference method (the table values of $|t|$ used for different confidence levels were: $t_{.95} = 2.31$, $t_{.96} = 2.51$, and $t_{.99} = 3.36$ for 8 degrees of freedom. All table values of F were at 95% confidence level).

was that for certain data pairs, the calculated t-value was greater than the critical table value of $|t|$ at 95% confidence level, and therefore 96% or 99% confidence levels were selected for complex real samples where appropriate.

The RDX contents of other military-purpose composite explosives (not shown in Table 2) using the proposed method were found as: Comp A5: 96.3%, Comp C4: 89.2%, Hexal P30: 67.6%, against the declared values as: Comp A5: 99%, Comp C4: 91% and Hexal P30: 67.5%, generally in accordance with each other.

From synthetic mixtures of TNT and RDX in the concentration range of 10–30 mg L^{-1} of each, having TNT/RDX (w/w) ratios of 1/3, 1/2, 2/3, 1, 2, and 3; the recoveries tested with the proposed method for TNT and RDX were in the range 92–101% and 97–104%, respectively (table not shown). From synthetic mixtures of TNT and HMX in the concentration range of 10–30 mg L^{-1} of each, having TNT/HMX (w/w) ratios of 1/3, 1/2, 2/3, 1, 2, and 3; the recoveries tested with the proposed method for TNT and HMX were in the range 102–104% and 94–104%, respectively (table not shown), showing quantitative recoveries for both types of mixtures. Given the fact that the two most commonly found explosives in environmentally contaminated sites are TNT and RDX, and that current U.S.-EPA methodology for their detection utilizes reverse-phase HPLC which is laboratory-limited, costly and laborious, often requiring pre-concentration of analytes, the proposed methodology may be a suitable alternative [27].

3.4. Semi-quantitative determination of nitro-aromatics on paper sensor

The reflectance of colored spots formed on reagent A-impregnated chromatographic paper was read within an optimal time period of 2–5 min (longer standing caused weakening of sensitivity) at the most homogeneous parts of the spots for optimal reproducibility. The calibration equations within the linear concentration range of nitro-aromatics using the paper sensor were given for 10–50 mg L^{-1} initial concentrations of TNT as:

$$R = 3.6 \times 10^{-3} C_{\mathrm{TNT}} + 4.79 \times 10^{-2}\ (r = 0.9927)$$

where R is the reflectance measured at 546 nm of TNT samples at C_{TNT} initial concentration (in mg L^{-1}). For the same initial concentration range, the calibration line for tetryl was:

$$R = 2.9 \times 10^{-3} C_{\mathrm{Tetryl}} + 2.47 \times 10^{-2}\ (r = 0.9924)$$

where R is the reflectance measured at 538 nm of tetryl samples at C_{Tetryl} initial concentration (in mg L^{-1}). The RSD (%) of TNT and tetryl determinations were in the range 3.9–10.4% and 3.6–18.0%, respectively.

The possibility of using the reagent A-impregnated chromatographic paper sensor after keeping in the dark in a vacuum dessicator was also investigated. Identical concentrations (10–50 mg L^{-1}) of TNT and tetryl were tested to yield the following calibration lines for TNT and tetryl:

$$R = 2.1 \times 10^{-3} C_{\mathrm{TNT}} + 4.91 \times 10^{-2}\ (r = 0.9992)$$

$$R = 3.1 \times 10^{-3} C_{\mathrm{Tetryl}} + 2.84 \times 10^{-2}\ (r = 0.9953)$$

Sensitivity was partly lost for TNT determinations on dessicator-kept paper sensors. Unfortunately, reagent B-impregnated chromatographic paper could not be used as sensor for nitramine-explosives due to high blank values; therefore, nitramine sensing should be made by application of fresh solutions of sample and successive reagents A & B on a non-impregnated paper.

In this paper spot detection of TNT samples, the reflectance maximum at 546 nm did not shift within the spectra (recorded for every minute) taken between the first and tenth minute. Likewise, the reflectance maxima of the spots at 559 nm and 547 nm for RDX and HMX, respectively, did not shift among the second and tenth minute spectra (1-min interval spectra not shown). These findings indicate that the color formation was rapid (average time: 2 min) and the once-formed colors were stable (up to the tenth minute), making paper spot sensing suitable for quick decision-making criminological applications possibly including airport security, because quantitative evaluation is not initially necessary for such screening purposes.

4. Conclusions

The requirement for rapid and cost-effective chemical sensing of explosive residues in soil and post-blast debris for environmental and criminological purposes is a critical issue for public health and security. The proposed reflectometric sensing method capable of quantitatively determining 0.1–0.5 μg-amounts of nitrated explosives on PVC tablets offers a simple, portable, and practical alternative that can be adapted for field use. This method can also be used for qualitative identification on chromatographic paper, as the successively sprayed reagents produce persistent colored spots (e.g., purplish brown for TNT, orange for tetryl, greenish blue for DNT, and pink for RDX and HMX) which can also be used for semi-quantitative analysis of nitro-aromatics. While the Expray qualitative test kit, the cheapest of similar analytical methods, utilizes a price less than 3 USD per test [7], the present method, capable of quantitative determination in addition to qualitative detection, has a chemicals (i.e. reagents + PVC for tablet) cost ≤10 cents per sample (excluding the instrument and operator costs). The developed method is believed to be useful in analyzing large number of samples at low cost from contaminated and remediated sites, and from criminological sites for quick decision making and field screening of police laboratories.

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