

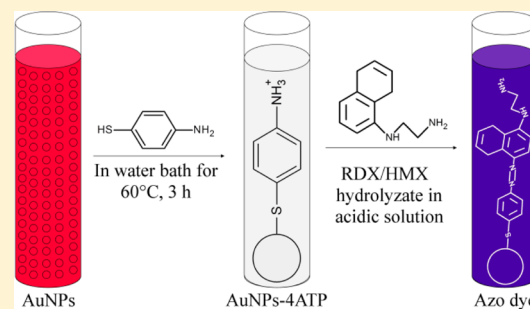
4-Aminothiophenol Functionalized Gold Nanoparticle-Based Colorimetric Sensor for the Determination of Nitramine Energetic Materials

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S Supporting Information

ABSTRACT: The heterocyclic nitramine compounds, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), are two most important military-purpose high explosives. Differentiation of RDX and HMX with colorimetric methods of determination has not yet been made because of their similar chemical structures. In this study, a sensitive colorimetric method for the determination of RDX and HMX was proposed on the basis of differential kinetics in the hydrolysis of the two compounds (yielding nitrite as a product) followed by their colorimetric determination using 4-aminothiophenol (4-ATP) modified gold nanoparticles (AuNPs) and naphthyl-ethylene diamine (NED) as coupling agent for azo-dye formation, abbreviated as “4-ATP–AuNP+NED” colorimetric method. After alkaline hydrolysis in a 1 M Na₂CO₃ + 0.04 M NaOH mixture solution at room temperature, only RDX (but not HMX) was hydrolyzed to give a sufficient colorimetric response in neutralized solution, the molar absorptivity (ϵ) at 565 nm and the limit of detection (LOD) for RDX being $(17.6 \pm 1.3) \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.55 \mu\text{g mL}^{-1}$, respectively. On the other hand, hot water bath (at 60 °C) hydrolysis enabled both nitramines, RDX and HMX, to give substantial colorimetric responses; i.e., ϵ and LOD for RDX were $(32.8 \pm 0.5) \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.20 \mu\text{g mL}^{-1}$ and for HMX were $(37.1 \pm 2.8) \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $0.24 \mu\text{g mL}^{-1}$, respectively. Unlike other AuNP-based nitrite sensors in the literature showing absorbance quenching within a relatively narrow concentration range, the developed sensor operated with an absorbance increase over a wide range of nitrite. Synthetic mixtures of (RDX + HMX) gave additive responses, and the proposed method was statistically validated against HPLC using nitramine mixtures.



The cyclic nitramine explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) are widely used as part of high-performance explosives such as plastic bonded explosives.^{1,2} HMX has nearly replaced its homologue RDX in importance and is more prevalent in recently manufactured explosive devices. The weapon system employed on antitank ranges contains a nominal 70% HMX and 30% TNT formulation with significant amounts of RDX present as an HMX production impurity.³ Reaction yield in RDX production using the conventional Bachmann process is 80–85% by weight, and the end product contains 10–17% (w/w) HMX.⁴ Thus, simple, low-cost, and robust methods of determination for separately quantitating RDX and HMX on-site and in-field are necessary. The requirements for quick decision making in criminology laboratories or reclaimed military sites often impose the use of simple and selective field techniques such as colorimetry and spectrophotometry. Considerably less attention has been devoted to field screening for RDX than for TNT, because RDX is known not to respond to either TNT colorimetric or enzyme immunoassay (EIA)-based screening methods.⁵ The most reliable colorimetric assay in literature, developed by Jenkins and Walsh⁶ for field testing of RDX residues, suffers from the nonquantitative reduction of RDX to nitrite upon

rapid contact of RDX containing acetone-soil extract with Zn dust and acetic acid (the nitrous acid being subsequently determined by the Griess reaction) and from the unstable character of HNO₂ in acidic medium, possibly reflected in the discrepancies reported by these authors between the colorimetric and HPLC assay results of RDX-contaminated soil samples.

Our research group has colorimetrically determined RDX by the simple Berhelot reaction after (Zn + HCl) reduction.⁷ Very few studies have been reported in the literature for RDX determination with nanoparticles, such as the surface plasmon resonance (SPR) reflectance spectroscopic detection of RDX by bisaniline-cross-linked AuNPs.⁸ The instability of high explosives to hydrolysis under distinctly alkaline conditions is well-known.⁹ Energetic materials, such as nitrate esters, nitroaromatics, and nitramines, usually decompose to form nitrates, nitrites, ammonia, nitrogen, hydrogen, organic acids, and formaldehyde. Several researchers have investigated the kinetics of alkaline hydrolysis of nitro-explosives such as RDX, HMX, TNT, and nitrocellulose.^{10–16} It has been reported that,

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in the presence of free OH^- ions, alkaline hydrolysis of RDX is 10 times faster than HMX.¹⁰ Thus, hydrolysis conditions should first be optimized to distinguish RDX from HMX. Focusing on nitrite as the major detectable constituent among the alkaline degradation products, the targets of this study were first to develop an AuNP-based colorimetric sensing method for nitramine assay and, second, to differentiate RDX and HMX with respect to their alkaline hydrolysis kinetics. To our best knowledge, no study has ever been reported to utilize AuNPs in a colorimetric assay of nitramines and to individually quantify RDX and HMX in complex matrices.

For colorimetric nitrite detection with AuNP probes, Daniel et al. had to prepare two different types of AuNPs with specific functionalization (i.e., containing thiolated precursors of aniline and naphthylamine derivatized NPs) which complicated the analytical procedure.¹⁷ When 4-aminothiophenol (4-ATP)-modified Au nanorods (AuNRs) were reacted with nitrite, the initially formed diazonium salt was deaminated by heating (at 95 °C) in EtOH solution, leading to the reduction of surface charges of AuNRs followed by their aggregation, producing a colorimetric response (i.e., absorbance decrease) for NO_2^- .¹⁸ Both AuNP-based colorimetric sensors operated on the basis of cross-linking or coagulation in the presence of nitrite, resulting in absorbance quenching within a narrow concentration range (i.e., varying between 20 and 30 μM NO_2^-).^{17,18} Thus, unlike these AuNP-based nitrite sensors existing in literature showing absorbance quenching within a relatively narrow concentration range of analyte, one of the aims of this work was to develop a AuNP sensor operating with absorbance increase (i.e., free from the interference of a high initial background) over a wide range of nitrite levels.

■ EXPERIMENTAL SECTION

Safety Note. RDX and HMX are highly energetic explosives and have a risk of explosion as a result of exposure to shock, friction, impact, fire, and electrostatic charge that may lead to detonation, deflagration, and violent burning reactions. The user must also avoid ingestion or inhalation of the toxic particles or vapors of these explosives. Stock solutions in appropriate solvents such as acetonitrile and acetone should be used in laboratory studies and storage.

Instrumentation and Chemicals. All reagents were analytical reagent grade unless otherwise stated. *N*-(1-Naphthyl)-ethylenediamine dichloride (NED) was purchased from Fluka, 4-aminothiophenol (4-ATP) (synthesis grade) from Merck, sulphanilic acid from Sigma-Aldrich, and all other reagents from Merck, Sigma-Aldrich, and Fluka. RDX and HMX were provided by Mechanical and Chemical Industry Corporation (MKEK) of Turkey.

The spectra and absorption measurements were recorded in matched Helma quartz cuvettes using a Varian CARY Bio 100 UV-vis spectrophotometer. For studying hydrolysis kinetics, a Daihan Scientific WiseBath water bath was used. For validation of the proposed assay in the determination of RDX and HMX against HPLC, a Perkin-Elmer Series 200 HPLC chromatographic instrument equipped with a UV (254 nm) detector was used.

Preparation of Solutions. The working solutions of RDX at 10–50 mg L^{-1} and HMX at 2.5–25 mg L^{-1} were prepared from the corresponding stock solutions of 500 mg L^{-1} concentration in 1:1 (v/v) acetone–water, the solvent medium also being useful for HPLC analysis. Hydrolysis mixture solution (1.0 M Na_2CO_3 and 0.04 M NaOH), HCl solutions

(1.0 and 5.0 M), H_3PO_4 solution (1.0 M), NED (20 mM), and 1% trisodium citrate solution (w/v) were all prepared in pure distilled water and 4-ATP (10 mM) solution in absolute ethanol.

Griess reagent was prepared by equally mixing 0.1% NED solution (prepared in distilled water) with 1% sulphanilic acid (prepared in 5% *ortho*-phosphoric acid aqueous solution). For validation purposes, 30 mg L^{-1} RDX and 15 mg L^{-1} HMX solutions were analyzed by the proposed method and by HPLC.¹⁹

Preparation of 4-ATP Functionalized AuNPs. AuNPs were synthesized by a previously published classical citrate reduction method.^{20,21} Briefly, 50 mL of 0.002% HAuCl_4 was heated to boiling. To this solution, 0.5 mL of 1% trisodium citrate was added. The solution was heated until its color change to red-wine, then removed from the hot plate, and cooled to room temperature.

AuNPs were further functionalized in the same solution (i.e., without centrifugation) with 4-ATP. First, the pH of AuNPs was adjusted to 3.0 where the thiol group of 4-ATP showed maximum affinity toward the surface of AuNPs. This acidic AuNPs solution was mixed with 10 mM 4-ATP at 8:1 (v/v) ratio and then stirred at 60 °C for 3 h. The solution was then left to stabilize at room temperature for 48 h. After functionalization, 4-ATP–AuNP remained useful for quantitative analysis for at least 2 days without losing its maximal absorbance against nitrite derived from nitramine hydrolysis.

Proposed Method for RDX and HMX Assay. The assay consisted of two parts, namely, hydrolysis of RDX/HMX and spectrophotometric determination of the hydrolyzate formed from RDX/HMX. The hydrolysis of RDX/HMX consisted of alkaline hydrolysis into NO_2^- , whereas spectrophotometric determination of the formed NO_2^- involved measurement by either Griess method²² or 4-ATP–AuNP+NED method.

Hydrolysis of RDX and HMX. A volume of 2.0 mL of hydrolysis solution (1.0 M Na_2CO_3 and 0.04 M NaOH mixture at final concentration) was added to 2.0 mL of RDX solution at 10–50 mg L^{-1} or HMX solution at 2.5–25 mg L^{-1} concentration and allowed to stand for 30 min for NO_2^- formation. RDX solutions were hydrolyzed enough at room temperature to respond to subsequent spectrophotometric tests, whereas HMX solutions could only produce detectable signals after hydrolysis at 60 °C on a water bath. Therefore, hydrolysis was performed on RDX-containing solutions both at room temperature and at 60 °C, while HMX solutions were only hydrolyzed at 60 °C.

Griess Method. Hydrolytic efficiencies of RDX and HMX were tested by the Griess method (see Supporting Information). At the end of the hydrolysis period, the alkaline sample was neutralized with 0.5 mL of 1.0 M HCl + 0.5 mL of 5.0 M HCl (the latter added in 0.1 mL portions so as to prevent instantaneous pH drops that may cause local enrichment and subsequent degradation of nitrous acid) before applying the Griess method.²² To 1.0 mL of this solution was added 3.0 mL of Griess reagent, and the absorbance of the final solution at 540 nm was read against a reagent blank.

4-ATP–AuNP+ NED Method. After RDX or HMX hydrolysis, the alkaline sample was neutralized with 0.5 mL of 1.0 M HCl + 0.6 mL of 5.0 M HCl (the latter added in 0.1 mL portions so as to prevent instantaneous pH drops that may cause local enrichment and subsequent degradation of nitrous acid). One mL of 4-ATP–AuNP was added, the reaction medium was adjusted to pH \approx 2 with 1.0 mL of 1.0 M H_3PO_4 ,

and then, 0.5 mL of 20.0 mM NED was added in this order. After 30 min, the absorbance at 565 nm was recorded against a reagent blank. The scheme for the 4-ATP–AuNP+NED method is summarized as: take RDX/HMX hydrolyzate; add 0.5 mL of 1.0 M HCl + 0.6 mL (in 0.1 mL portions) of 5.0 M HCl + 1.0 mL of 4-ATP–AuNP + 1.0 mL of 1.0 M H_3PO_4 (final pH \approx 2) + 0.5 mL of 20.0 mM NED in this order; measure A_{565} against a reagent blank after 30 min of NED addition.

Determination of RDX/HMX in Complex Materials.

Assay in Synthetic (RDX + HMX) Mixtures. The room temperature–hydrolysis assay was applied to synthetic mixtures of (RDX + HMX) by maintaining a constant concentration of RDX at 20 mg L^{-1} and varying HMX concentration at RDX/HMX ratios (w/w) of 1:1, 1:2, 1:5, and 1:10 in 1:1 (v/v) acetone–water. On the other hand, the 60 °C water bath–hydrolysis assay was applied to synthetic mixtures by either fixing RDX at 2.5 mg L^{-1} and varying HMX at 1:1, 1:2, 1:5, and 1:10 (w/w) RDX/HMX or fixing HMX at 2.5 mg L^{-1} and varying RDX at 1:1, 1:2, 1:5, and 1:10 (w/w) HMX/RDX. Loamy clay soil artificially contaminated with RDX and HMX individually and in admixture (RDX and HMX content: 250 $\mu\text{g g}^{-1}$ each) was extracted with acetone using the procedure given by Jenkins and Walsh,⁶ and the findings of the proposed and reference (HPLC) methods were compared (see Supporting Information).

Assay in the Presence of Common Soil Ions. RDX (30 mg L^{-1}) and HMX (15 mg L^{-1}) were separately assayed in the presence of 10-fold (Ca^{2+} , Mg^{2+}) and 50-fold (K^+ , SO_4^{2-} , Cl^- , NO_3^-) concentrations of common ions. All monovalent ions and Ca^{2+} were tested in 1:1 (v/v) acetone– H_2O mixture solution, except Mg^{2+} and SO_4^{2-} which were tested in 1:4 (v/v) acetone– H_2O mixture solution. In order to eliminate Mg^{2+} interference on RDX recovery, loamy clay soil artificially contaminated with 30 ppm RDX alone or in admixture with 300 ppm Mg^{2+} was subjected to acetone leaching,⁶ and the results obtained with the proposed method were compared (see Supporting Information).

Statistical Analysis. Descriptive statistical analyses were performed using Excel software (Microsoft Office 2010) for calculating the means and the standard error of the mean. Results were expressed as the mean \pm standard deviation (SD). Method validation against HPLC determination of explosives was made by means of Student (*t*-) and *F*-tests of statistical analysis.

RESULTS AND DISCUSSION

Nitrite (produced from alkaline hydrolysis of nitramines) reacts with 4-ATP–AuNP to give a diazonium salt which couples to NED to form the chromophore of the surface-modified gold nanoparticle assay, as shown in Figure 1.

Under acidic conditions, the amine groups of 4-aminothiophenol–modified AuNPs are converted into the cationic conjugate acid (ammonium) form, which stabilize the nanoparticles by electrostatic repulsion. In the presence of nitrite (emerging as a product of nitramine hydrolysis) and mineral acids, this primary aromatic amine turns into a diazonium salt. Although diazonium salts are not stable in aqueous solution because of the tendency to lose diazonium groups,¹⁸ they were stabilized via azo-dye formation by the addition of NED, causing a bathochromic shift in the localized SPR absorption band of AuNPs as a result of charge-transfer interaction at nanoparticle surfaces.²³ Surface complexation between an

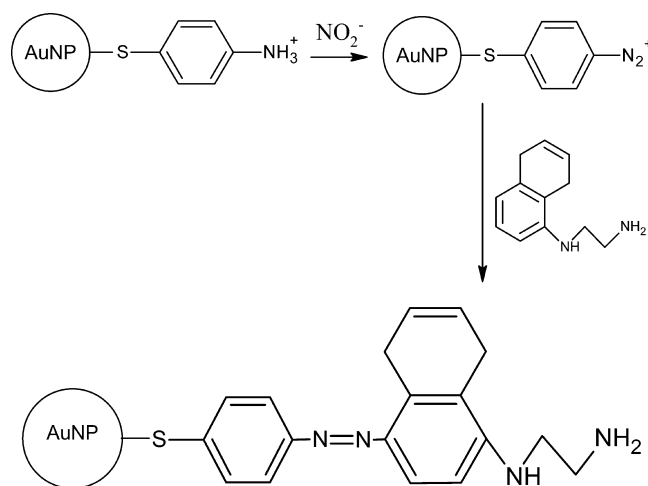


Figure 1. Schematic presentation of NO_2^- (produced from alkaline hydrolysis of RDX or HMX) determination with 4-ATP modified gold nanoparticles and NED coupling agent.

organic molecule possessing an active functional group and AuNPs was reported to play an important role in both the stabilization and new chromophore formation of nanoparticles.²⁴ The proposed (4-ATP–AuNP+NED) method was shown to be superior to the application of (4-ATP+NED) binary combination without involvement of AuNPs, due to its much improved sensitivity and linearity with respect to nitramine concentration (see Supporting Information).

Analytical Figures of Merit. RDX solutions at final concentrations ranging between 2.63 and 13.16 mg L^{-1} gave a linear calibration curve as a result of room temperature hydrolysis:

$$A_{565} = (7.94 \pm 0.57) \times 10^{-2} C_{\text{RDX}} - (7.22 \pm 5.1) \times 10^{-2} \\ (N = 5, r = 0.9992)$$

where the molar absorptivity for RDX was $\epsilon = (17.6 \pm 1.3) \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$, with a limit of detection (LOD) = 0.55 mg L^{-1} and a limit of quantification (LOQ) = 1.83 mg L^{-1} (LOD = $3\sigma_{\text{bl}}/m$ and LOQ = $10\sigma_{\text{bl}}/m$, where σ_{bl} denotes the standard deviation of a blank and *m* is the slope of calibration curve for spectrophotometric RDX determination).

Three readings were made for each concentration, and the relative standard deviation (RSD) of a given set of readings varied in the range of 0.6–1.4% as a result of room temperature hydrolysis, depending on RDX concentration. On the other hand, RDX solutions at final concentrations ranging between 0.66 and 6.58 mg L^{-1} gave the following calibration curve when the hydrolysis reaction was carried out in a water bath at 60 °C:

$$A_{565} = (1.48 \pm 0.03) \times 10^{-1} C_{\text{RDX}} - (5.05 \pm 1.3) \times 10^{-2} \\ (N = 5, r = 0.9999)$$

where the molar absorptivity for RDX was $\epsilon = (32.8 \pm 0.5) \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$, with a limit of detection (LOD) = 0.20 mg L^{-1} and a limit of quantification (LOQ) = 0.67 mg L^{-1} , RSD varying between 2.6% and 6.2%, depending on the concentration. The ϵ value was almost doubled with elevated temperature hydrolysis of RDX, due to greater nitrite production. The spectra of underivatized and modified AuNPs and end products with the hydrolyzate of RDX at different concentrations are shown in Figure 2.

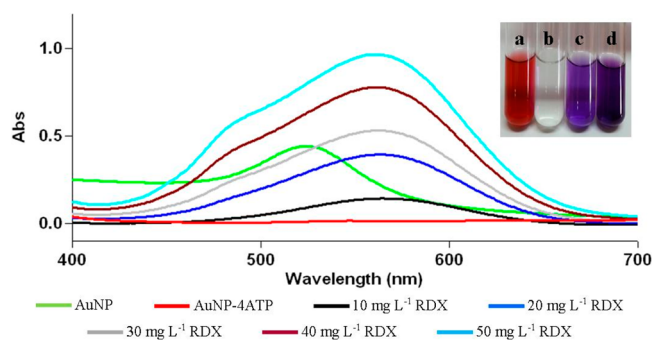


Figure 2. Spectra of underivatized AuNP, AuNP modified with 4-ATP, and end products, obtained from RDX hydrolysis at room temperature in the concentration range of 10–50 mg L⁻¹. (The color images of the test tubes containing (a) AuNP, (b) 4-ATP–AuNP, (c) 20 mg L⁻¹ RDX (end product), and (d) 50 mg L⁻¹ RDX (end product) is shown in the inset figure.)

HMX solutions at final concentrations ranging between 0.66 and 6.58 mg L⁻¹ gave a linear calibration curve as a result of 60 °C hydrolysis in a water bath:

$$A_{565} = (1.25 \pm 0.1) \times 10^{-1} C_{\text{HMX}} + (4.95 \pm 4.0) \times 10^{-2} \\ (N = 5, r = 0.9991)$$

where the molar absorptivity for HMX was $\epsilon = (37.1 \pm 2.8) \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$, with a limit of detection (LOD) = 0.24 mg L⁻¹, limit of quantification (LOQ) = 0.80 mg L⁻¹, and RSD in the range of 3.7–7.7%, depending on the concentration. The spectra of underivatized and modified AuNPs and end products with the hydrolyzate of HMX at different concentrations are shown in Figure 3.

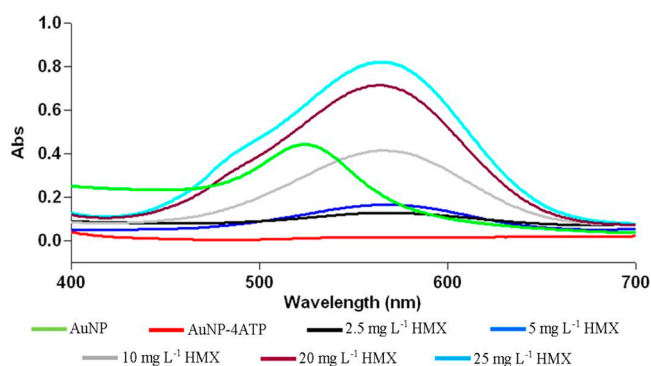


Figure 3. Spectra of underivatized AuNP, AuNP modified with 4-ATP, and end products, obtained from HMX hydrolysis at 60 °C, in the concentration range of 2.5–25 mg L⁻¹.

The coefficients of variation (CVs) of intra-assay measurements for RDX (at room temperature) and HMX were 1.9% and 2.4%, respectively, whereas those of interassay measurements were 3.6% and 3.1%, respectively ($N = 5$).

Hoffsommer and co-workers²⁵ reported that RDX degradation in alkaline medium was initiated by a single denitration step to first form pentahydro-3,5-dinitro-1,3,5-triazacyclohex-1-ene, which then hydrolyzed further leading to rapid ring cleavage and decomposition. While only a few studies have been conducted with HMX, it has been shown that HMX undergoes alkaline hydrolysis at a slower rate than RDX,^{10,26} to

give a product distribution (composed of NO_2^- , N_2O , NH_3 , N_2 , and HCOOH) similar to that obtained with RDX.¹³

Under alkaline conditions, RDX was hydrolyzed both at room temperature and at 60 °C, whereas HMX showed negligible hydrolysis at room temperature and only produced a substantial signal upon 60 °C hydrolysis. Thus, mixtures of RDX and HMX can be analyzed with the proposed method initially by performing room temperature hydrolysis and calculating the concentration of RDX component using the relevant calibration curve for RDX and then by exploiting the principle of additivity of absorbances (in conformity with Beer's law) upon 60 °C hydrolysis by subtracting the RDX contribution from the overall absorbance measured at 565 nm and using the calibration equation for HMX at the same temperature.

RDX/HMX mixtures having compositional ratios between 1:10 and 10:1 (w/w) were assayed using the proposed colorimetric procedure, and the A_{565} values of the resulting solutions were collected in Tables 1 and 2, with mention of expected contribution from each component. Data in Table 1 show that, except at very high (10:1) concentration relative to RDX, HMX showed negligible absorbance after room temperature hydrolysis and that RDX could be assayed alone without contribution from HMX. On the other hand, after 60 °C hydrolysis, the absorbances originating from RDX and HMX were additive, with overall recoveries laying in the range of 97.6–109.3% (Table 2).

Common ions abundantly found in soil and groundwater at 10-fold (Ca^{2+} , Mg^{2+}) and 50-fold (K^+ , SO_4^{2-} , Cl^- , NO_3^-) concentrations of RDX did not affect the recoveries (96–99%), except Mg^{2+} (62%) tested at 30 mg L⁻¹ RDX. For HMX, the same ions at identical ratios did not affect the recoveries (96–102%) tested at 15 mg L⁻¹ HMX. In the presence of 10-fold Mg^{2+} , RDX recovery from acetone-leached soil was $94 \pm 5\%$ when the recovered RDX was hydrolyzed at room temperature (recovery was quantitative after 60 °C hydrolysis). Due to its extremely low solubility in acetone, magnesium interference could be completely eliminated with acetone leaching.

HPLC Determination of RDX and HMX for Method Validation. RDX and HMX working solutions in acetone–water at 10–50 mg L⁻¹ and 5–25 mg L⁻¹ concentrations, respectively, were individually analyzed with HPLC, and the mean values of three repetitive injections were used for calculations. The calibration equations between peak area and concentration were:

$$\text{peak area} = 3.79 \times 10^4 C_{\text{RDX}} + 4.69 \times 10^4 \quad (r = 0.9994)$$

$$\text{peak area} = 2.5 \times 10^4 C_{\text{HMX}} + 2.0 \times 10^4 \quad (r = 0.9993)$$

The proposed spectrophotometric method for RDX and HMX were validated against the reference HPLC procedure¹⁹ using standard solutions of 30 mg L⁻¹ RDX and 15 mg L⁻¹ HMX in acetone–water on $N = 5$ repetitive analyses, essentially showing no significant differences between the precision and accuracy of results (Table 3). The t - and F -tests were used for comparing the population means and variances, respectively.²⁷ The confidence levels used in validation of RDX findings were 95% and 99%, respectively, for t - and F -tests, and were 95% for HMX using both t - and F -tests (Table 3). The proposed method was statistically validated against HPLC using synthetic mixtures of nitramines (i.e., solutions containing 10 mg L⁻¹ RDX and 10 mg L⁻¹ HMX). No significant differences were observed after application of (t -) and F -tests (Table 3). After

Table 1. Additivity of Absorbances of RDX and HMX with Respect to 4-ATP–AuNP Colorimetry in Synthetic Mixture Solutions (after alkaline hydrolysis at room temperature and neutralization)

final concn. of mixture soln.	mixing ratio (w/w)	absorbance (% of expected value)
5.26 mg L ⁻¹ RDX + 5.26 mg L ⁻¹ HMX	1:1 RDX/HMX	0.334 ((99.1 ± 3.0)%)
5.3 mg L ⁻¹ RDX		0.325
5.3 mg L ⁻¹ HMX		0.012
5.26 mg L ⁻¹ RDX + 10.53 mg L ⁻¹ HMX	1:2 RDX/HMX	0.343 ((102.4 ± 3.0)%)
5.26 mg L ⁻¹ RDX		0.325
10.53 mg L ⁻¹ HMX		0.010
5.26 mg L ⁻¹ RDX + 26.32 mg L ⁻¹ HMX	1:5 RDX/HMX	0.354 ((102.2 ± 3.0)%)
5.26 mg L ⁻¹ RDX		0.325
26.5 mg L ⁻¹ HMX		0.021
5.26 mg L ⁻¹ RDX + 52.63 mg L ⁻¹ HMX	1:10 RDX/HMX	0.404 ((105.3 ± 5.0)%)
5.26 mg L ⁻¹ RDX		0.325
52.63 mg L ⁻¹ HMX		0.058

Table 2. Additivity of Absorbances of RDX and HMX with Respect to 4-ATP–AuNP Colorimetry in Synthetic Mixture Solutions (after alkaline hydrolysis at 60 °C and neutralization)

final concn. of mixture soln.	mixing ratio (w/w)	absorbance (% of expected value)
0.66 mg L ⁻¹ RDX + 0.66 mg L ⁻¹ HMX	1:1 RDX/HMX	0.208 ((102.5 ± 2.6)%)
0.66 mg L ⁻¹ RDX		0.132
0.66 mg L ⁻¹ HMX		0.076
0.66 mg L ⁻¹ RDX + 1.32 mg L ⁻¹ HMX	1:2 RDX/HMX	0.306 ((109.3 ± 0.3)%)
0.66 mg L ⁻¹ RDX		0.132
1.32 mg L ⁻¹ HMX		0.148
0.66 mg L ⁻¹ RDX + 3.29 mg L ⁻¹ HMX	1:5 RDX/HMX	0.494 ((97.0 ± 0.6)%)
0.66 mg L ⁻¹ RDX		0.132
3.29 mg L ⁻¹ HMX		0.378
0.66 mg L ⁻¹ RDX + 6.58 mg L ⁻¹ HMX	1:10 RDX/HMX	0.921 ((107.8 ± 0.6)%)
0.66 mg L ⁻¹ RDX		0.132
6.58 mg L ⁻¹ HMX		0.723
0.66 mg L ⁻¹ HMX + 1.32 mg L ⁻¹ RDX	1:2 HMX/RDX	0.336 ((97.6 ± 2.2)%)
0.66 mg L ⁻¹ HMX		0.128
1.32 mg L ⁻¹ RDX		0.216
0.66 mg L ⁻¹ HMX + 3.29 mg L ⁻¹ RDX	1:5 HMX/RDX	0.697 ((97.9 ± 1.0)%)
0.66 mg L ⁻¹ HMX		0.128
3.29 mg L ⁻¹ RDX		0.569
0.66 mg L ⁻¹ HMX + 6.58 mg L ⁻¹ RDX	1:10 HMX/RDX	1.212 ((100.2 ± 0.9)%)
0.66 mg L ⁻¹ HMX		0.128
6.58 mg L ⁻¹ RDX		1.082

Table 3. Statistical Comparison of the Proposed Method with HPLC for the Determination of RDX and HMX

analyte	method	mean conc.	SD (σ)	$S^{a,b}$	$t^{a,b}$	t_{table}^b	F^b	F_{table}^b
RDX	proposed method (spectrophotometric)	32.45	0.625					
	HPLC-UV detection	32.31	0.190	0.462	0.465	2.306	10.843	15.98
HMX	proposed method (spectrophotometric)	14.614	0.350					
	HPLC-UV detection	14.848	0.205	0.286	1.292	2.306	2.916	6.39
RDX ^c	proposed method (spectrophotometric)	9.624	0.165					
	HPLC-UV detection	9.588	0.071	0.127	0.531	2.306	5.438	6.39
HMX ^d	proposed method (spectrophotometric)	10.371	0.474					
	HPLC-UV detection	10.351	0.305	0.286	0.399	2.306	2.425	6.39

^a $S^2 = \{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2\}/(n_1 + n_2 - 2)$ and $t = (\bar{a}_1 - \bar{a}_2)/\{S(1/n_1 + 1/n_2)^{1/2}\}$, 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, $n_1 = n_2 = 5$. ^bStatistical comparison made on paired data produced with proposed and reference methods; the results given only on the row of the reference method. ^cRDX component in {10 mg L⁻¹ RDX and 10 mg L⁻¹ HMX} mixture. ^dHMX component in {10 mg L⁻¹ RDX and 10 mg L⁻¹ HMX} mixture.

acetone leaching of nitramine-contaminated soil, the recovery of RDX component (from RDX + HMX mixture) with the proposed method was found to be $97 \pm 5\%$ and of HMX component was $92 \pm 4\%$, whereas the corresponding

percentages found with HPLC were $96 \pm 4\%$ for RDX and $93 \pm 3\%$ for HMX.

CONCLUSIONS

Noble metal nanoparticle-based colorimetric methods of explosive detection are usually criticized because of their low selectivity and sensitivity in spite of their speed, low-cost, and simplicity. One of the nanoparticle-based sensing mechanisms that enables the transduction of chemical binding events into optical signals based on changes in the localized surface plasmon resonance (LSPR) extinction or shifts in LSPR λ_{max} of AuNPs is the charge-transfer interaction at nanoparticle surfaces.²³ Thus, this work has optimized the differential hydrolysis of nitramines into nitrite (where the more stable nitro-aromatic explosives like TNT are not appreciably hydrolyzed; see Supporting Information) and developed a selective nitrite sensor utilizing the interaction of LSPR absorption of AuNPs through 4-ATP functionalization with diazo-coupling by NED on the NPs surface. Unlike its literature counterparts showing an absorbance decrease (due to cross-linking or coagulation of NPs) over a narrow range of nitrite concentrations,^{17,18} this sensor showed an absorbance increase over nearly one order-of-magnitude levels of nitrite emerging from nitramine hydrolysis. Moreover, the sensor distinguishes RDX from HMX via their differential kinetics in hydrolysis, which has not been colorimetrically accomplished before. Again, unlike similar colorimetric sensing methods, the 4-ATP–AuNP+NED sensor is capable of detecting less than 1 $\mu\text{g mL}^{-1}$ of RDX and HMX simultaneously at a most suitable wavelength in the visible range (i.e., at 565 nm showing a red-shift with respect to underivatized AuNPs). The proposed method was validated for nitramine mixtures. This AuNP-based selective and sensitive detection/quantification of nitramines is believed to be beneficial for in-field/on-site analysis of postblast debris in crime scene investigations and environmental sampling (e.g., in contaminated land and groundwater remediation). Utilization of chemical sensing methods (having low cost and high selectivity) in screening analysis enables the investigation of large areas so as to focus on the spots of high contamination which can later be sampled for in depth off-site analysis,²⁸ and this type of screening may greatly reduce analysis costs in monitoring nitro explosives-contaminated land remediation.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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