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Spectrophotometric and chromatographic determination of insensitive energetic materials: HNS and NTO, in the presence of sensitive nitro-explosives

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ABSTRACT

As there are no molecular spectroscopic determination methods for the most widely used insensitive energetic materials, 2,2′,4,4′,6,6′-hexanitrostilbene (HNS) and 3-nitro-1,2,4-triazole-5-one (NTO), in the presence of sensitive nitro-explosives, two novel spectrophotometric methods were developed. For HNS and TNT mixtures, both analytes react with dicyclohexylamine (DCHA) forming different colored charge-transfer complexes, which can be resolved by derivative spectroscopy. The spectrophotometric method for NTO measures the 416-nm absorbance of its yellow-colored Na^+NTO^- salt formed with NaOH. TNT, if present, is pre-extracted into IBMK as its Meisenheimer anion forming an ion-pair with the cationic surfactant cetyl pyridinium (CP^+) in alkaline medium, whereas the unextracted NTO is determined in the aqueous phase. The molar absorptivity (ε, L mol^{-1} cm^{-1}) and limit of quantification (LOQ, mg L^{-1}) are as follows: for HNS, $\varepsilon = 2.75 \times 10^4$ and LOQ = 0.48 (in admixture with TNT); for NTO, $\varepsilon = 6.83 \times 10^3$ and LOQ = 0.73. These methods were not affected from nitramines and nitrate esters in synthetic mixtures or composite explosives. The developed methods were statistically validated against HPLC, and the existing chromatographic method was modified so as to enable NTO determination in the presence of TNT. These simple, low-cost, and versatile methods can be used in criminology, remediation/monitoring of contaminated sites, and kinetic stability modeling of munitions containing desensitized energetic materials.

1. Introduction

Energetic materials are extensively used for both civil and military applications. Since the storage and transport of conventional sensitive explosives like RDX, HMX, and TNT have given rise to unforeseen accidents, the strategic demand for insensitive energetic materials is on the rise. Enhanced performance and reduced sensitivity combining good thermal stability with shock-impact resistance are required in modern ammunition. New generation explosive formulations bear desensitizers so as to increase the shock resistance while maintaining good thermal properties and high energy capacity. Hexanitrostilbene (HNS) is a common example of this group of heat resistant explosives [1]. HNS is practically insensitive to electrostatic spark, less sensitive to impact than tetryl, and radiation resistant [2]. It proved its efficiency as a component of heat-resistant formulations employed in the Apollo spaceship, in seismic experiments on the moon and for use in achieving stage separation in space rockets [3]. As the most studied nitrotriazole explosive, NTO was reported by Becuwe and Delclos [4] to be far less sensitive than RDX and HMX. It is more stable than TNT and RDX, but its sensitivity to ignition is slightly higher than that of TNT. NTO alone or in conjunction with either RDX or HMX are used as fillings for insensitive munitions: IM [3,5] which respond less violently to accidental environmental stimuli.

Most studies on insensitive energetic materials are generally associated with their synthesis, characterization, thermal stability and degradation, principally aiming at 'chemical purity' analysis. Starting from 1980s, the analysis of more complex mixtures was initiated. Kayser used DMSO as solvent for HPLC separation of polynitro-compounds on a RP C-18 radial-pak cartridge with isocratic elution using MeOH–H_2O, and was able to completely resolve HNS and TNT peaks in the chromatogram [6]. Oehrle, who accomplished the analysis of NTO along with 14 other nitramine and nitroaromatic explosives by micellar electrokinetic capillary chromatography, has claimed that current HPLC methods give reasonable results for the synthetic mixtures of NTO with TNT, which are not so easily amenable to other composite explosives due to the pH required to resolve NTO [7]. Le Campion et al. were able to separate NTO and its biodegradation metabolites with the aid of

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HPLC on a Hypercarb column packed with porous graphite carbon, using UV detection at 220 nm [8].

The requirements for quick decision making and screening analyses in criminology laboratories or reclaimed military sites often impose the use of cost-effective, non-laborious, simple, selective, and yet sensitive field techniques such as colorimetric and extractive-spectrophotometric determination. Such inexpensive and practical spectrophotometric methods that can determine HNS and NTO with reasonable selectivity have not yet been developed. HNS was spectrophotometrically determined without selectivity by Glover and Kayser at the Naval Ordnance Laboratory with the use of its charge-transfer (CT) bands at 460 and 510 nm with 1.5–3.0 M ethylenediamine (EDA), however, tri-, tetra-, and hexanitro aromatic explosives structurally similar to HNS gave close absorption bands [9]. In the search of selective CT reagents for HNS, we explored dicyclohexylamine (DCHA) which was previously shown by our research group to enable a sensitive and selective spectrophotometric determination of TNT [10]. In preliminary experiments, we saw that DCHA (that gave a purple color with TNT) [10] gives an orange-colored CT complex with HNS.

The known composite explosives containing NTO and TNT include 50% NTO–50% TNT known as TNTO, 65% NTO–35% TNT known as GD-1, and 40% NTO–60% TNT known as South African 1 [11]. Currently, none of these composite explosives can be spectrophotometrically analyzed to differentiate between constituents. NTO can form a yellow-colored salt – by losing one or two protons – in aqueous solution upon reaction of its —NH protons with NaOH, and the NTO^{2-} salt absorbs light at 416 nm due to intramolecular charge transfer [12]. At this alkalinity, TNT forms a Meisenheimer anion [13] which can be extracted with CP^+ cationic surfactant into isobutyl methyl ketone (IBMK) [14] having a characteristic absorption at 461 nm. Thus, this extractive separation from alkaline solution formed the basis of the developed spectrophotometric method for separate NTO and TNT estimation. The developed spectrophotometric methods for the simultaneous determination of NTO and TNT were validated against an established HPLC method [8] which was further modified in this work to enable an efficient separation of these two analytes. Both the developed spectrophotometric method and HPLC method were applied to complex mixtures containing these analytes.

2. Materials and methods

2.1. Chemicals

All reagents were analytical reagent grade unless otherwise stated. Sodium methyl 4-hydroxybenzoate (sodium methylparaben) and dicyclohexylamine (DCHA) were purchased from Fluka, cetylpyridinium bromide (CP^+Br^-) from E. Merck, sodium hydroxide from Riedel-de Haën, and all other reagents from E. Merck, Sigma–Aldrich and Fluka. HNS and NTO were provided by Middle East Technical University (METU) and Sabancı University, respectively, as originally synthesized products in the corresponding chemistry department laboratories. Certified standard material HNS and 2-amino-4,6-dinitrotoluene (2-ADNT) were purchased from AccuStandardand and Dr. Ehrenstorfer, respectively. Nitro- and composite explosives were provided by Machinery & Chemistry Industries Institution (MKEK) of Turkey under a mutual research project.

2.2. Solutions

The working solutions of HNS at 3–30 mg L^{-1} and NTO at 2.2–22 mg L^{-1} were prepared from the corresponding stock solutions of 500 mg L^{-1} concentration in pure acetone (diluent was acetone for HNS and 1:1 (v/v) acetone – water for NTO). For HPLC analysis, acetone was the preferred solvent for HNS and NTO analyzed in acetone–water medium. Sodium methylparaben was prepared at 0.1% in acetone–water. NaOH and CP^+Br^- were prepared at 5% and 7.5×10^{-3} M, respectively, in water.

2.3. Instruments

The ^1H and ^{13}C NMR spectra of the synthesized HNS sample were recorded on a Bruker DPX 400 spectrometer. For the developed analytical methods, spectrophotometric measurements were made with the aid of a Varian Cary UV-Vis Spectrophotometer (scan rate = 600 nm min^{-1}, and spectral resolution = 1 nm), working either in the main or first-derivative spectral mode. For validation of the proposed assay for HNS and NTO against HPLC, a Thermo Fisher and Perkin Elmer Series 200 HPLC chromatographic instrument equipped with a UV detector was used.

2.4. Recommended procedure for HNS assay (single and simultaneous determination with TNT)

To 4 mL of the standard HNS solution (without TNT) containing 3–30 mg L^{-1} HNS in acetone were added 1 mL of 0.1% (w/v) sodium methyl paraben solution in acetone–water, and 1 mL of DCHA (final solution contained 2–20 mg L^{-1} HNS). After waiting for 30 min, the absorbance of the red-violet charge-transfer complex was measured in a stoppered quartz cell at a wavelength of 464 nm against a reagent blank.

Synthetic mixtures of HNS and TNT containing both constituents at mass concentration ratios of HNS:TNT = 1:1, 1:2, 1:5, 1:10, 2:1, 5:1, and 10:1 were prepared, and the derivative spectrophotometric assay for the determination of both constituents was applied. Since the main spectra of HNS and TNT overlapped, a wavelength difference ($\Delta\lambda$) of 5 nm was taken for first derivative (^1D) spectra. In ^1D spectra, peak-to-zero method was used at 528 nm for HNS and at 485 nm for TNT. Since the ^1D signal of TNT provided higher sensitivity at 550 nm (at which HNS shows little derivative absorbance), TNT could be determined at both wavelengths (*i.e.*, 485 and 550 nm), preferentially at the latter wavelength in accordance with the principle of additivity of derivative absorbances. The calibration line for HNS (as 1st derivative absorbance at 528 nm *versus* concentration) was redrawn for 2–20 mg L^{-1} HNS solutions containing a fixed concentration (10 mg L^{-1}) of TNT; the LOQ value of HNS was found by processing these data.

2.5. Recommended procedure for NTO assay

To 5 mL of the standard NTO solution in 1:1 (v/v) acetone–water were added 0.5 mL of 5% aqueous NaOH, 2 mL of 7.5×10^{-3} M aqueous CP^+Br^- solution, and 7.5 mL of IBMK; the test tube was stoppered, and slowly agitated (upside down) 10 times at room temperature. The volume of the aqueous phase after separation of phases was approximately 5.5 mL. The aqueous phase containing yellow-colored Na^+NTO^- salt was filtered off through a double-fold blue band quantitative filter paper, and the absorbance at 416 nm was read against a reagent blank. The calibration line was drawn for 2–20 mg L^{-1} concentrations of NTO in final aqueous solution. The organic phase was kept for the assay of TNT when present.

2.6. Validation of the proposed derivative-spectrophotometric HNS assay against HPLC

Validation of the proposed spectrophotometric HNS assay against HPLC was carried out with 2–20 mg L^{-1} solutions. With the exception of mobile phase composition, the literature HPLC conditions [6] were used: Supelco Discovery C_{18} (5 μm),

250 mm × 4.6 mm ID reversed phase (RP)-column conjunction with a UV (254 nm) detector; the injection volume was 10 μL. Instead of the mobile phase of the literature method (*i.e.*, MeOH:H_2O (1:1, v/v)), 50% AcCN + 50% H_2O (v/v) mixture was used at a flow rate of 1 mL min^{-1} to enable separation of the *cis*- and *trans*-isomers of HNS.

Statistical comparisons between the findings of the recommended (derivative-spectrophotometric) and reference (HPLC) methods were made with the aid of *t*- and *F*-tests.

2.7. Validation of the proposed NTO assay against HPLC

For validation of the proposed assay against HPLC on a 2:1 (w/w) NTO–TNT synthetic mixture sample, NTO and TNT solutions at 5–50 mg L^{-1} and 2–20 mg L^{-1} in acetone:water, respectively, were used. The reference HPLC method [8] capable of separating NTO from related 1,2,4-triazole-3-one derivatives was modified for analyzing NTO and TNT simultaneously. An HPLC chromatographic instrument equipped with a Thermo Hypercarp (7 μm) 100 mm × 4.6 mm ID porous graphitic carbon (PGC)-column was used in conjunction with a UV (220 nm) detector. The Hypercarb column was eluted with a gradient: $t_{0–5\,min}$: 100% A (30% AcCN and 0.05% TFA in water), $t_{5–5.5\,min}$: 100% B (50% AcCN, 25% MeOH, 25% THF), $t_{5.5–15.5\,min}$: 100% B at a flow rate of 1 mL min^{-1}. The injection volume was 20 μL. Statistical comparisons between the findings of the recommended (spectrophotometric) and reference (HPLC) method – together with the modification of the latter – were made with the aid of *t*- and *F*-tests.

2.8. Determination of HNS in explosive mixtures

HNS-CompB and HNS-Octol mixtures were prepared at 1:1, 1:2, 1:5, 1:10, 2:1, 5:1, and 10:1 HNS:TNT concentration ratios (*i.e.*, with respect to the TNT content of the mentioned composite explosives), and the proposed assay for HNS was applied. Separation of HNS-DCHA and TNT-DCHA charge-transfer complexes was carried out by derivative spectrophotometry in synthetic HNS–TNT mixtures.

Binary and ternary mixtures of nitro-explosives, namely RDX, HMX and PETN, were prepared at 150 mg L^{-1} concentration (*i.e.*, 10-fold of analyte), and HNS was added at a final concentration of 15 mg L^{-1}, followed by the application of the proposed assay for HNS. Since derivative spectrophotometry was unnecessary for HNS determination in the presence of nitramines and nitrate esters, the absorbance of the HNS-DCHA charge-transfer complex was measured at a wavelength of 464 nm against a reagent blank.

The widely found metabolites of TNT in the environment are listed as 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitroluene (4-ADNT) [15] due to bacterial reduction of one nitro to amino group. Therefore, in this work, the possible interference of 2-ADNT to the determination of HNS in admixture with TNT and DNT was investigated in synthetic mixtures containing 10 mg L^{-1} HNS, 10 mg L^{-1} TNT, 20 mg L^{-1} 2-ADNT, and 500 mg L^{-1} DNT prepared in acetone–water (1:1, v/v).

2.9. Determination of NTO in complex materials

(i) Assay in TNT mixtures or in TNT-containing composite explosives: synthetic mixtures of NTO and TNT at NTO:TNT concentration ratios (w/w) of 1:1, 1:2, 1:5, 1:10, 2:1, 5:1, and 10:1 were prepared in 1:1 (v/v) acetone–water, and the proposed assay for NTO was applied. Absorbances were measured at 416 nm for NTO left in the aqueous phase, and at 461 nm for TNT extracted into the organic phase. NTO–CompB and NTO–Octol mixtures were prepared at 1:1, 1:2, and 2:1 NTO:TNT concentration ratios (calculated on the basis of the TNT content of composite explosives), and were treated as above. Binary and ternary mixtures of nitro-explosives, namely RDX, HMX and PETN, were prepared at 110 mg L^{-1} concentration (*i.e.*, 10-fold of analyte), NTO was added at a final concentration of 11 mg L^{-1}, and were treated as above. It should be noted that solvent extraction is unnecessary in the absence of nitro-aromatics yielding colored Meisenheimer complexes.

(ii) Assay in the presence of common soil ions: NTO (10 mg L^{-1}) was assayed in the presence of 50-fold concentrations of common ions. The potential interferent ions (Cl^-, SO_4^{2-}, NO_3^-, Mg^{2+}, Ca^{2+}, K^+, Cu^{2+}) were tested in 1:1 (v/v) acetone–H_2O mixture solution.

2.10. Statistical analysis

Descriptive statistical analyses were performed using Excel software (Microsoft Office 2003) for calculating the means and the 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

3.1. Structural characterization of the synthesized HNS

HNS was prepared according to Shipp-Kaplan method [15]. The NMR spectra were recorded in d6-DMSO (1H NMR: d6-DMSO $\delta = 2.5$), d6-DMSO (13C NMR: $\delta = 40$). Chemical shifts (δ) were reported relative to TMS as the internal standard. The synthesized HNS material behaved in all respects as the standard material [16] in regard to ^1H and ^{13}C NMR, FTIR, DSC, and TGA data.

3.2. Nature of interaction as the basis of spectrophotometric analyses

In general, the intensely colored product formed between aromatic $-NO_2$ compounds and amine type Lewis bases may be attributed to the partial transfer of electronic charge (*i.e.*, through overlap of orbitals of appropriate symmetry) from the Lewis base to the aromatic nucleus of the nitro-compound depleted off electron density, owing to the electron-attracting behavior of the $-NO_2$ substituents. The charge-transfer (CT) interaction, named after Mulliken [17,18], occurs *via* partial transfer of charge from *n*-lone pair of the amine to the oxygen-π^* of the nitro-group [19]. Modern understanding of CT interaction involves the partial transfer of charge from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO) of the CT complex upon light absorption. Although polynitro-aromatics are well-known to form anionic σ-complexes, the so-called Meisenheimer complexes [13] with hydroxide or amines, such Meisenheimer complexes are so high in free energy that they possibly make up only a very small fraction of the full equilibrium population. Thus, since the deprotonation equilibria of nitro-aromatics in the presence of Brønsted bases lie far on the side of the unreacted nitro-aromatics, preventing the detection by NMR of the deprotonated minority species that gives the solutions their strong color, alternative deprotonated structures have recently been suggested to be mainly responsible for the observed colors of these complexes, specifically for DNT-alkylamine interactions in DMSO [20]. In this work, the nature of interaction between HNS and DCHA is presumed to be of CT type (*i.e.*, transfer of electronic charge – upon incident light – from the amine donor to HNS acceptor) as shown

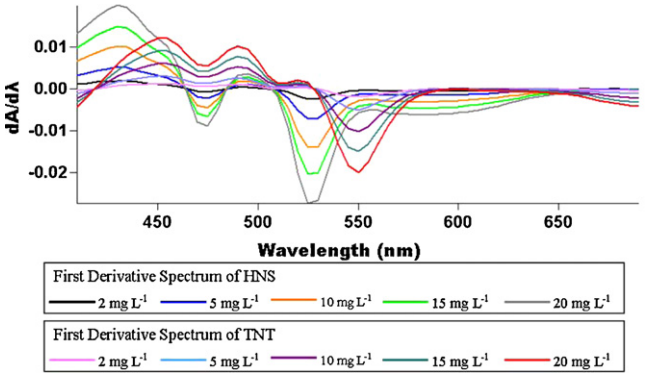


Fig. 1. First derivative spectra of HNS and TNT.

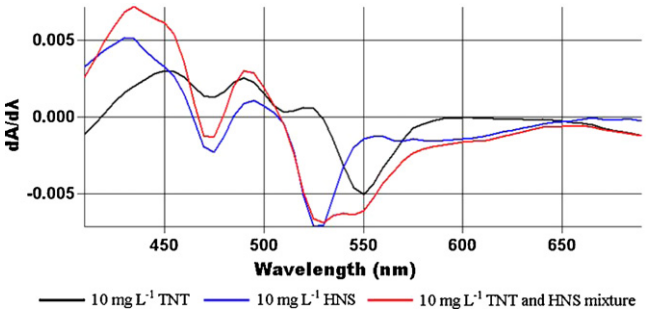
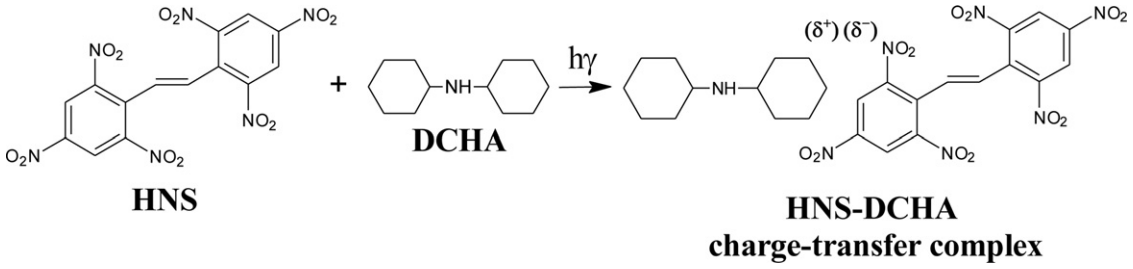


Fig. 2. The ^{1}D spectra of 10 mg L^{-1} HNS and 10 mg L^{-1} TNT individually and in admixture.

in Eq. (1).



(1)

by multiplying C_{HNS} with its derivative absorptivity coefficient at 550 nm.

3-Nitro-1,2,4-triazol-5-one (NTO) loses protons stepwise in alkaline medium, first from the more acidic 4-NH, and then from the less acidic 1-NH group, forming mono- and di-anions, respectively. The long-wave electronic $\pi \rightarrow \pi^*$ transition in NTO mono- and di-anions is one-electron and occurs between the HOMO and LUMO levels. Analysis of the electron density distribution for these π-molecular orbitals shows that the first $\pi \rightarrow \pi^*$ transition involves its redistribution between the $C^3N^4C^5{=}O$ moiety, N^1 atom, and nitro group, which is accompanied, in particular, by a decrease in the charge on oxygen [12]. The 416-nm peak of NTO forming the basis of its colorimetic determination possibly arises from this HOMO → LUMO transition of the yellow-colored di-anion showing a high conductivity in water (Singh et al. [21]) (and therefore a high affinity toward the polar water molecules *via* ion–dipole interactions, hindering its extractability into organic solvents).

3.3. Derivative-spectrophotometric determination of HNS and TNT in admixture

HNS and TNT give orange and violet colored charge-transfer complexes with DCHA, respectively. As the main charge-transfer spectra of these two nitro-explosives significantly overlap, first-derivative (^{1}D) spectra (Fig. 1) were drawn to identify peak-to-zero and other analytical wavelengths so as to devise derivative-spectrophotometric methods for the quantification of HNS and TNT in mixtures.

By definition of derivative absorbance, $^1D = \partial A/\partial\lambda \approx \Delta A/\Delta\lambda$ by keeping $\Delta\lambda$ as small as possible. When the first-derivative (^{1}D) spectrum is recorded with wavelength intervals ($\Delta\lambda$) of 5 nm, HNS and TNT can be determined at 528 and 485 nm, respectively, using the peak-to-zero method. Alternatively at 550 nm where TNT shows strong first-derivative absorption (^{1}D) and HNS shows little interference, the amount of TNT in a binary mixture can be estimated by subtracting the ^{1}D value of HNS from that of the mixture. Naturally, the concentration of HNS constituent in the mixture can be accurately estimated from a calibration line of $^1D_{528\,nm}$ *versus* concentration, and therefore the $^1D_{550\,nm}$ contribution of HNS to the total ^{1}D measured at the same wavelength can be calculated

Fig. 2 depicts the first-derivative spectra of 10 mg L^{-1} HNS and 10 mg L^{-1} TNT individually and in admixture, showing 528 and 485 nm as the peak-to-zero wavelengths for the two analytes, and 550 nm as a sensitive analytical wavelength for TNT with little ^{1}D contribution from HNS. It is also visible from Fig. 2 that the ^{1}D absorbances of mixture constituents (*i.e.*, TNT and HNS) are additive at 550 nm.

For the determination of HNS as the single analyte, the main (rather than derivative) absorbance at 464 nm can be used in accordance with Beer's law. HNS solutions at final concentrations ranging between 2 and 20 mg L^{-1} gave a calibration curve:

$$A_{464\,nm} = 6.1 \times 10^{-2} C_{HNS} - 8 \times 10^{-3}$$

where the molar absorptivity for HNS was $\varepsilon = 2.75 \times 10^4$ L mol^{-1} cm^{-1}, with a limit of detection (LOD) = 0.08 mg L^{-1} and a limit of quantification (LOQ) = 0.26 mg L^{-1} (by definition, LOD = $3\sigma_{bl}/m$ and LOQ = $10\sigma_{bl}/m$, where σ_{bl} denotes the standard deviation of a blank, and m is the slope of the calibration curve for spectrophotometric HNS determination).

Using the first-derivative absorbances (^{1}D) at 528 nm, a linear calibration between 2 and 20 mg L^{-1} HNS was possible with the equation:

$$^1D_{528\,nm} = 1.35 \times 10^{-3} C_{HNS} - 9.76 \times 10^{-6}$$

with a linear correlation coefficient of $r = 0.9997$.

Three readings were made for each concentration, and the relative standard deviation (RSD, %) of a given set of readings varied in the range 0.4–2.9% for HNS, depending on the concentration.

The calibration line for HNS was redrawn for 2–20 mg L^{-1} HNS solutions containing a fixed concentration (10 mg L^{-1}) of TNT, with the following equation:

$$^1D_{528\,nm} = 1.31 \times 10^{-3} C_{HNS} - 3.41 \times 10^{-5} \ (r = 0.9999)$$

yielding an LOQ value of 0.48 mg L^{-1}. This calculated LOQ value was tested in a synthetic mixture solution of (0.5 mg L^{-1}

Table 1
Recovery (%) of HNS and TNT from nitro-explosive mixtures (mean of 3 repetitive measurements for each sample).

Explosive mixture	Using first-derivative absorbance of HNS[c]	Using first-derivative absorbance of TNT[c]	
	Recovery (%) at $^1D_{528}$	Recovery (%)[d] at $^1D_{485}$	Recovery (%) at $^1D_{550}$
HNS–TNT[a,e]	93 ± 3.5 to 104 ± 0.6	20 ± 2 to 105 ± 5.3	95 ± 2.7 to 105 ± 2.7
HNS-CompB[a,f] (HNS with TNT constituent of CompB)	92 ± 2.2 to 103 ± 0.6	20 ± 5.8 to 82 ± 2.2	98.5 ± 0.9 to 107 ± 5.6
HNS-Octol[a,e] (HNS with TNT constituent of Octol)	97 ± 3.5 to 106 ± 0.4	30 ± 5.8 to 99 ± 1.3	95 to 105 ± 5
Binary and ternary mixtures of HNS with other nitro-explosives, (*i.e.*, RDX, HMX and PETN)[b,g]		96 ± 0.8 to 99 ± 0.4	

[a] Seven mixtures of HNS and TNT ranging between 1:10 and 10:1 (w/w) concentration ratios.
[b] Six different mixtures containing 10-fold (w/w) nitro-explosive interferents.
[c] Recoveries were expressed as (mean % ± RSD); higher precisions (*i.e.*, lower relative standard deviations) were noted with higher ratios of the concerned analyte in the mixture.
[d] Recoveries as low as 20% were noted due to the low sensitivity for TNT at this wavelength.
[e] Binary mixtures containing 5 mg L^{-1} + 5 mg L^{-1}, 5 mg L^{-1} + 10 mg L^{-1}, 2 mg L^{-1} + 10 mg L^{-1}, 2 mg L^{-1} + 20 mg L^{-1}, 10 mg L^{-1} + 5 mg L^{-1}, 10 mg L^{-1} + 2 mg L^{-1}, and 20 mg L^{-1} + 2 mg L^{-1} of HNS and TNT, respectively.
[f] Binary mixtures containing 6.5 mg L^{-1} + 6.5 mg L^{-1}, 6.5 mg L^{-1} + 13 mg L^{-1}, 3 mg L^{-1} + 15.8 mg L^{-1}, 2 mg L^{-1} + 20.2 mg L^{-1}, 13 mg L^{-1} + 6.5 mg L^{-1}, 13 mg L^{-1} + 2.6 mg L^{-1}, and 20.08 mg L^{-1} + 2.08 mg L^{-1} of HNS and TNT, respectively.
[g] Binary and ternary mixtures containing 10 mg L^{-1} HNS and 100 mg L^{-1} RDX, HMX and/or PETN each, respectively (recovery (%) calculated using the main absorbance of HNS at 464 nm).

HNS + 10 mg L^{-1} TNT), and the experimentally measured 1st derivative absorbance (^1D) for HNS was 0.0006.

For the determination of TNT as the single analyte, the main (rather than derivative) absorbance maximum at 528 nm can be used in accordance with Beer's law:

TNT solutions at final concentrations ranging between 2 and 20 mg L^{-1} gave a calibration curve:

$$A_{528\,nm} = 5.2 \times 10^{-2} C_{TNT} - 1.66 \times 10^{-3}$$

where the molar absorptivity for TNT was $\varepsilon = 1.19 \times 10^4$ L $mol^{-1} cm^{-1}$, with LOD 0.019 mg L^{-1} and LOQ 0.064 mg L^{-1}.

The first derivative absorbances (^1D) at 485 nm, where a linear calibration between 2 and 20 mg L^{-1} TNT was possible, gave the equation:

$$^1D_{485\ nm} = 4.48 \times 10^{-4} C_{TNT} - 2.44 \times 10^{-5}$$

with a linear correlation coefficient of $r = 0.9997$. However, since the derivative absorptivity at this peak-to-zero wavelength is quite low (*i.e.*, at the order of 10^{-4}), the TNT constituent of a binary mixture can be more sensitively quantified at 550 nm. The first derivative absorbances (^1D) for TNT solutions in the range of 2 and 20 mg L^{-1} gave the equation:

$$^1D_{550\,nm} = 1.0 \times 10^{-3} C_{TNT} - 9.8 \times 10^{-6}$$

with a linear correlation coefficient of $r = 0.9998$.

3.4. Derivative-spectrophotometric determination of HNS and TNT in nitro-explosive mixtures

Explosive mixtures of HNS with TNT, in the presence of RDX, HMX, PETN, CompB and Octol as potential interferents, were analyzed at various concentration ratios of HNS-to-TNT between 1:10 and 10:1 (w/w). The percentage recoveries of HNS and TNT from these explosive mixtures are depicted in Table 1. The sensitivity problem encountered for ^1D of TNT at 485 nm yielded low recoveries (Table 1) down to 20% (Table 1). The recoveries using the first-derivative absorbances at 528 nm and 550 nm for HNS and TNT, respectively, were almost quantitative (*i.e.*, close to 100%) for both explosives (Table 1), confirming the non-interference of other nitro-explosives (RDX, HMX and PETN) to the proposed method.

2-ADNT (up to 20 mg L^{-1} concentration) had neither main nor derivative absorbance at $\lambda \geq 485$ nm (covering the three analytical wavelengths of the proposed derivative spectroscopic method), and did not interfere with the determination of HNS, TNT, and DNT in synthetic mixtures containing 10 mg L^{-1} of the first two constituents and 500 mg L^{-1} of DNT. However, at higher mass ratios of ADNT to other constituents, possible intermolecular attractions between $-NH_2$ of ADNT and $-NO_2$ groups of the other energetic substances gave rise to deviations in additivity of derivative absorbances from the expected values. Thus, the tolerance level of ADNT to the determination of HNS in admixture with TNT and DNT was 20 mg L^{-1}.

Table 2
Recovery (%) of NTO and TNT from nitro-explosive mixtures.

Explosive mixture	Recovery (%) of NTO[a] at A_{416}	Recovery (%) of TNT[a] at A_{461}
NTO–TNT[b] (mixtures at 1:10–10:1 (w/w) concentration ratios)	95 ± 1.9 to 99 ± 5.2	95 ± 2.9 to 99.6 ± 1.0
NTO–CompB[c] (mixtures at 1:2–2:1 concentration ratios of NTO-to-TNT in CompB)	99 ± 1.2 to 101 ± 2.1	98 ± 0.8 to 101 ± 5.9
NTO–Octol[c] (mixtures at 1:2–2:1 concentration ratios of NTO-to-TNT in Octol)	98 ± 1.4 to 105 ± 1.0	97 ± 2.2 to 105 ± 0.8
Binary and ternary mixtures of NTO with nitramines and nitrate esters, namely RDX, HMX and PETN, at 10-fold analyte concentrations[d]	95 ± 0.9 to 97 ± 1.2	

[a] Recoveries were expressed as (mean % ± RSD); higher precisions (*i.e.*, lower RSDs) were noted with higher ratios of the concerned analyte in the mixture.
[b] Binary mixtures containing 10 mg L^{-1} + 10 mg L^{-1}, 5 mg L^{-1} + 10 mg L^{-1}, 2 mg L^{-1} + 10 mg L^{-1}, 2 mg L^{-1} + 20 mg L^{-1}, 12 mg L^{-1} + 6 mg L^{-1}, 10 mg L^{-1} + 2 mg L^{-1}, and 20 mg L^{-1} + 2 mg L^{-1} of NTO and TNT, respectively.

[c] Binary mixtures containing 10 mg L^{-1} + 10 mg L^{-1}, 5 mg L^{-1} + 10 mg L^{-1}, and 10 mg L^{-1} + 5 mg L^{-1} of NTO and TNT, respectively.
[d] Binary and ternary mixtures containing 10 mg L^{-1} NTO and 100 mg L^{-1} RDX, HMX and/or PETN each, respectively.

3.5. Simultaneous spectrophotometric determination of NTO and TNT

The developed spectrophotometric method for NTO determination in the presence of TNT is based on the extraction into isobutyl methyl ketone (IBMK) of the Meisenheimer anion of TNT (formed with aqueous NaOH) with cetylpyridinium (CP^+) surfactant cation [14]. During this procedure, the —NH group of NTO lying between two neighbor electron-withdrawing substituents (*i.e.*, carbonyl and nitro groups) and therefore having the stronger acidity should have formed a yellow-colored Na^+NTO^- salt with NaOH which stayed in the aqueous phase [21]. The NTO^- anion owes its color to intramolecular charge transfer, but since it is more hydrophilic than TNT Meisenheimer anion, it was not extracted with CP^+ into IBMK; another possibility is the formation of double-negative charged NTO-anion, which may not be extracted with organic solvents due to enhanced ion–dipole interactions with water molecules. Absorbances were measured at 416 nm for NTO and at 461 nm for TNT. The formation of the sodium-salt of NTO is expressed by Eq. (2) [21], and of the IBMK-extractable CP^+ ion-pair of TNT anion [14] by Eq. (3).

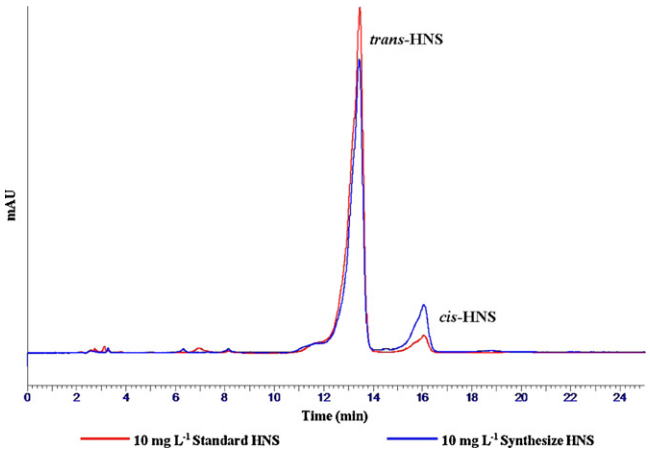
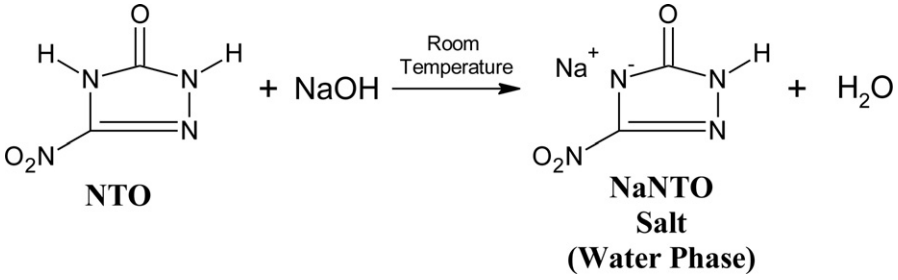
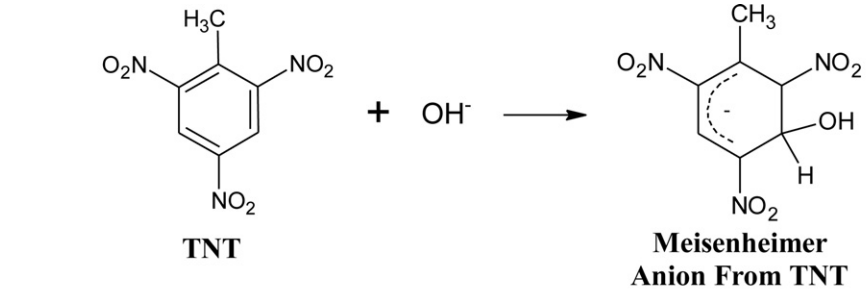


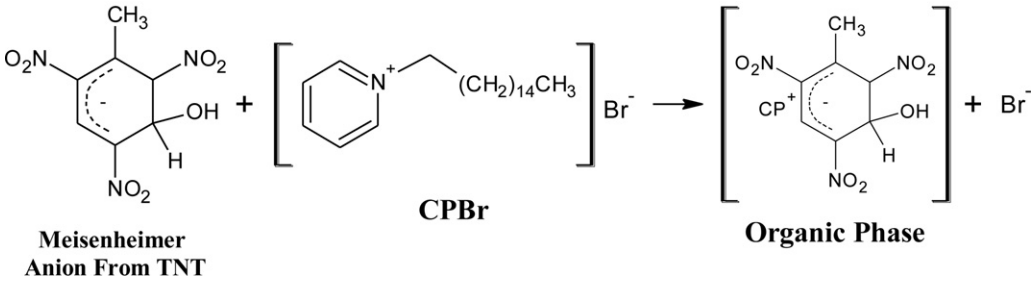
Fig. 3. The coincident chromatograms of standard and synthesized HNS samples.

TNT solutions at final concentrations in the range of $2-20\,mg\,L^{-1}$ (in the IBMK phase) gave a calibration curve:



(2)





(3)

NTO solutions at final concentrations (in aqueous phase) ranging between 2 and 20 mg L^{-1} gave a perfectly linear calibration curve drawn as 416-nm absorbance *versus* concentration:

$$A_{416\ nm} = 4.5 \times 10^{-2} C_{NTO} - 1.64 \times 10^{-2} \quad (r = 0.9999)$$

where the molar absorptivity for NTO was $\varepsilon = 6.83 \times 10^3\,L\,mol^{-1}\,cm^{-1}$, with LOD $0.22\,mg\,L^{-1}$ and LOQ $0.73\,mg\,L^{-1}$. Three readings were made for each concentration, and the relative standard deviation (RSD, %) of a given set of readings varied in the range 1.1–3.2% for NTO, depending on the concentration.

$$A_{461\ nm} = 5.0 \times 10^{-2} C_{TNT} - 1.26 \times 10^{-2} \quad (r = 0.9998)$$

where the molar absorptivity for TNT was $\varepsilon = 1.25 \times 10^4\,L\,mol^{-1}\,cm^{-1}$, with LOD $0.15\,mg\,L^{-1}$ and LOQ $0.50\,mg\,L^{-1}$.

3.6. Spectrophotometric determination of NTO and TNT in explosive mixtures

The percentage recoveries of NTO and TNT from explosive mixtures (Table 2) were close to 100% for both explosives, confirming

the non-interference of the nitro-explosives, *i.e.*, RDX, HMX and PETN, to the proposed method.

Common ions abundantly found in soil and groundwater (Cl^-, SO_4^{2-}, NO_3^-, Mg^{2+}, Ca^{2+}, K^+, and Cu^{2+}) at 50-fold concentrations of NTO did not affect the recoveries (97–99%), except Ca^{2+} (83%), tested at 10 mg L^{-1} NTO.

3.7. HPLC determination of HNS and NTO for method validation

The literature HPLC method was used for the determination of HNS [6] at 10 mg L^{-1} concentration, and the coincident chromatograms of standard and synthesized HNS samples are given in Fig. 3. Both samples essentially consist of the *trans*-HNS, with minor contribution from the *cis*-isomer (Fig. 3).

The calibration equation for the HPLC analysis of 2, 5, 10, 15, and 20 mg L^{-1} HNS solutions in acetone was:

$$\text{Peak Area} = 1.6 \times 10^5 C_{\text{HNS}} - 4.85 \times 10^4 \quad (r = 0.9995)$$

As for NTO, the literature method [8] was slightly modified for effective separation of NTO and TNT. For this purpose, a Hypercarb column was used having 100% porous graphitic carbon (PGC) spherical particles as the stationary phase. The surface of PGC is composed of flat sheets of hexagonally arranged carbon atoms as in a very large polynuclear aromatic molecule [22]. The retention mechanism of PGC differs from that of conventional reverse phase (RP) columns, as the graphitic surface interacts with the analyte through π interactions (explaining some strong interactions with certain solutes) and polar interactions with the lone-pair electrons available on the molecules. Stereochemical differences between the analytes can give rise to great differences in the strength of these interactions. Another well-known characteristic of PGC is its utility in separating isomers that are difficult to separate with conventional reversed phase columns [23]. The elution strength of organic solvents (from Hypercarb) is solute-dependent and an important tool to adjust retention and selectivity. In general, methanol (MeOH) and acetonitrile (AcCN) are similar in strength but weaker than 2-propanol (IPA) which in turn is weaker than either tetrahydrofuran (THF) or dichloromethane (DCM) [22]. Since TNT strongly interacts with the PGC surface *via* the π bonds in its aromatic structure, THF containing mobile phase was used as an ion-pairing agent for elution of TNT, resulting in improved resolution and peak shape. After adapting optimal chromatographic conditions, NTO was successfully separated from TNT (Fig. 4), though with higher background absorption with increasing retention time in accordance with gradient elution [8] on the side of the TNT peak, and the proposed spectrophotometric NTO assay was validated against the modified and reference (unmodified method) [8] HPLC methods.

NTO and TNT working solutions in acetone–water at 5–50 mg L^{-1} and 2–20 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:

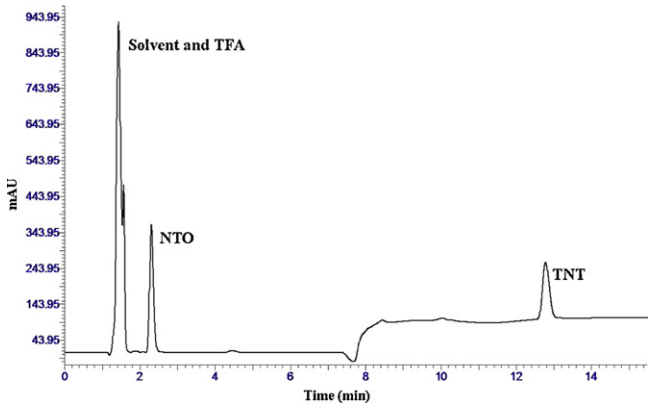


Fig. 4. Chromatogram of 50 mg L^{-1} NTO and 15 mg L^{-1} TNT mixture (the Hypercarb column was subjected to gradient elution, as described in the experimental section subtitled "*Validation of the proposed NTO assay against HPLC*").

$$\text{Peak Area} = 4.88 \times 10^4 C_{\text{NTO}} - 2.69 \times 10^4 \quad (r = 0.9999)$$

$$\text{Peak Area} = 1.33 \times 10^5 C_{\text{TNT}} - 3.54 \times 10^4 \quad (r = 0.9999)$$

For a fixed concentration of NTO at 10 mg L^{-1}, the calibration equation for TNT was:

$$\text{Peak Area} = 1.33 \times 10^5 C_{\text{TNT}} - 3.91 \times 10^4 \quad (r = 0.9999)$$

which was not essentially different from that of individual determinations (the RSD for NTO peaks was 1.0%).

For a fixed concentration of TNT at 15 mg L^{-1}, the calibration equation for NTO was:

$$\text{Peak Area} = 4.95 \times 10^4 C_{\text{NTO}} - 2.57 \times 10^4 \quad (r = 0.9999)$$

which was not essentially different from that of individual determinations (the RSD for TNT peaks was 1.4%).

The LOD for NTO was 0.82 mg L^{-1} (giving a HPLC peak area of 1.29×10^4). Since a peak corresponding to this amount of NTO (of retention time = 2.39 min) was barely visible in the normal chromatogram run up to 15 min (Fig. 4), a new chromatogram of 0.82 mg L^{-1} NTO with time scale up to 3 min was recorded (Fig. 5), distinctly showing the NTO peak (at 2.39 min) different from that of the solvent.

The proposed spectrophotometric methods for HNS and NTO were validated against their HPLC counterparts using standard

Table 3
Statistical comparisons of the proposed spectrophotometric methods with HPLC for the determination of HNS, NTO and TNT.

Analyte	Method	Mean conc.	SD (σ)	$S^{a,b}$	$t^{a,b}$	$t_{\text{table}}{}^b$	F^b	$F_{\text{table}}{}^b$
HNS	Proposed method (Spectrophotometric)	10.47	0.24	–	–	–	–	–
	HPLC–UV	10.41	0.13	0.192	0.490	2.306	3.304	6.39
NTO	Proposed method (Spectrophotometric)	9.72	0.23	–	–	–	–	–
	HPLC–UV	9.83	0.11	0.182	0.914	2.306	4.164	6.39
TNT	Proposed method (Spectrophotometric method for NTO)	4.85	0.29	–	–	–	–	–
	HPLC–UV	4.91	0.07	0.215	0.426	2.306	15.74	15.98
NTO[c]	Proposed method (Spectrophotometric)	10.35	0.27	–	–	–	–	–
	Reference method (HPLC–UV)	10.07	0.16	0.219	2.034	2.306	2.852	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, $n_1 = n_2 = 5$.
[b] Statistical comparison made on paired data produced with proposed and reference methods; the results given only on the row of the reference method.
[c] NTO was validated against reference method [8].

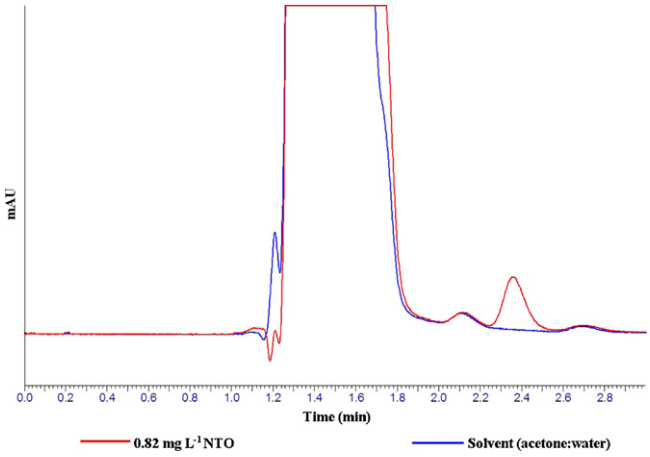


Fig. 5. The coincident chromatograms of 0.82 mg L^{-1} NTO (LOD value) and solvent (acetone–water); retention time of NTO = 2.39 min.

solutions of 10 mg L^{-1} HNS in acetone, (10 mg L^{-1} NTO + 5 mg L^{-1} TNT) mixture solution in acetone – water and only 10 mg L^{-1} NTO in 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 [24]. The confidence levels of validation were 95% for HNS and NTO using both *t*- and *F*-tests, and 95% for the t-test of TNT and 99% for the F-test of TNT (Table 3).

4. Conclusions

To fill in a literature gap regarding the requirement of simple, low-cost and versatile molecular spectroscopic analytical methods for insensitive energetic materials, two new spectrophotometric methods for the determination for HNS and NTO have been devised. HNS was determined by derivative-spectrophotometry in the presence of the strongest interferent, TNT, with the use of the charge-transfer complexes of both analytes with DCHA. In synthetic explosive mixtures where NTO was added as desensitizer to the sensitive TNT, TNT was determined in the organic phase by extraction into IBMK of the ion-pair formed from the cationic surfactant CP^+ and TNT-Meisenheimer anion in alkaline medium, whereas the unextracted NTO was determined in the aqueous phase as its yellow-colored salt. Both spectrophotometric methods (developed for HNS and NTO) were shown not to be affected by nitramines (RDX and HMX) and nitrate esters (PETN), tested in either synthetic explosive mixtures or composite explosives (CompB and Octol). The proposed methods were capable of quantifying HNS and NTO with LOQ values less than 1 mg L^{-1} for both analytes, and were statistically validated against HPLC; in addition, the existing chromatographic method for NTO was modified so as to enable its determination in the presence of TNT. The developed methods are 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. Moreover, since insensitive energetic materials are increasingly added to sensitive nitro-aromatics (TNT), nitramines (RDX and HMX), and nitrate esters (PETN) in desensitized explosives, the developed methods can be used for their low-cost and practical analysis during the whole shelf-life for differentiating between undegraded sensitive and insensitive components; thus, the kinetic stability of munitions containing desensitized energetic materials can be better modeled.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.12.077.

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