Fluorescence of Aromatic Amines and Their Fluorescamine Derivatives for Detection of Explosive Vapors

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Nitroaromatics (such as dinitrotoluene, trinitrotoluene, and nitrobenzene) found in explosive vapors from buried landmines can be reduced to aminoaromatics by a novel process involving Pd metal nanocatalysts prepared in supercritical fluid carbon dioxide and supported on multi-walled carbon nanotubes. These aminoaromatics are fluorescent and, if desired, the fluorescence yield can be increased and the fluorescence maxima shifted further toward the red by reaction with appropriate derivatizing agents such as fluorescamine. Corrected spectra for these chemicals and their derivatives are included. Subpicomolar detection limits have already been achieved using a laboratory spectrofluorometer with a 150 W Xe arc lamp. Using lasers as excitation sources, this approach has the potential for developing a field sensor competitive with other methods currently used for detecting explosive vapors from land mines.

Index Headings: **Aminoaromatics; Explosive vapors; Fluorescence; Nitroaromatics; Palladium nanoparticles.**

INTRODUCTION

Thousands of forgotten buried landmines exist all over the world. It is also common knowledge that plastic landmines and improvised explosive devices (IEDs) are causing many casualties in Iraq. Most of these mines contain TNT (2,4,6-trinitrotoluene). TNT is also commonly the analyte of most interest near munitions factories or storage areas. Some of the techniques for detection assume metallic mines or require very expensive instrumentation or are otherwise unreliable. The best detection, especially for plastic mines, involves detection of a mixture of vapors leaking from the mines. These are impurities or degradation products such as 2,4-dinitrotoluene (DNT),[1] which are more volatile than TNT by at least a couple of orders of magnitude. Other dinitrotoluenes or other aromatic nitrated species such as TNT, nitrotoluene, or nitrobenzene may be present, but can be handled similarly to the procedure described in this paper. For this application very low levels of vapors, lower than parts-per-trillion levels, must be measured. References on the chemistry of explosives and related field detection are included.[2–6] Several electronic "noses" using different techniques have also been proposed and perhaps the most promising is "FIDO" which uses fluorescent polymers developed by T. Swager[3] and incorporated in an electronic nose by Nomadics Company (Stillwater, OK). In this case the nitroaromatics such as DNT quench specially synthesized fluorescent polymers. Fluorescence quenching may not be specific enough for some forensic applications, and also the fluorescent polymer may not be reliable and reproducible enough, as well as being expensive to produce in bulk. In any case, after a reduction step, direct fluorescence of the aromatic amines or their derivatives provides an alternative approach, providing greater specificity, which might have advantages for certain applications. An environmental high-performance liquid chromatography (HPLC) method with fluorescence detection for aminoaromatics in soil has been reported.[7] This is a laboratory method and requires the use of more solvent. To the extent that we are talking about explosive vapors rather than soil or ground water samples, most matrix and quenching effects are eliminated. As stated in Guilbault,[8] aromatic nitro compounds are not good candidates for analysis by fluorescence spectroscopy unless they are converted chemically into fluorescent compounds with amino groups by chemical reduction. Fluorescence spectra of aminoaromatics have been reported in the literature.[8–10] Kasha[11,12] has discussed the theory of fluorescence for chemicals like the aromatic amines.

The current paper includes an updated, corrected set of fluorescence spectra using the purest aromatic amines available including aniline, o- and p-toluidines, 2,4- and 2,6-diaminotoluene, and 2,4,6-triaminotoluene. For the sort of mixture of explosive vapors in which we are most interested, 2,4,-DNT, which can be reduced to 2,4-DAT, has been reported as the major specie.[1] Fluorescent fluorescamine derivatives of aromatic amines are known[8,13] and we also include several examples for these aromatic amine derivatives. Recently, a Siberian group[14,15] proposed a scheme for reducing nitroaromatics to aminoaromatics as a similar way of measuring explosive vapors, but they used different reducing agents, included no spectra of the aminoaromatics, and gave only one example of a spectrum of a fluorescamine derivative. Our proposed technique uses catalysis by metallic (Pd) nanoparticles[16–18] synthesized in supercritical fluid carbon dioxide or water-in-hexane microemulsion and supported on carbon nanotubes to efficiently reduce the nitroaromatics to aminoaromatics, which are fluorescent themselves. UV-VIS fluorescence is the best and simplest ultratrace technique, being capable with laser excitation as an option of even single molecule detection. This fluorescence technique can be made extremely sensitive (fluorescence derivatization with reagents such as fluorescamine can make the derivatized amines even more highly fluorescent) and the fluorescence can be shifted further to the red where there is less background fluorescence. In this paper we discuss the fluorescence spectra and chemistry required as a framework for proving such a field sensor feasible. Initial tests were made with a spectrofluorometer (Spex FluoroMax-3™) having a 150 watt Xe arc lamp for tunability. A small laser may be substituted for extra sensitivity, once the desired wavelength (for the fluorescamine derivative of DAT at about 391 nm) is determined. These measurements are designed to show that direct detection of aromatic amines or their derivatives can provide an alternative approach for measuring explosives to fluorescence quenching of nitroaromatic components of explosives.

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EXPERIMENTAL

Instrumentation. Standard fluorescence measurements of the aminoaromatics and their derivatives with fluorescamine were used to generate excitation and emission spectra using a Spex (HORIBA Jobin Yvon) analytical grade spectrofluorometer (FluoroMax-3™) with a 150 watt Xe arc lamp. The instrument was calibrated by measuring the spectrum of the xenon lamp for the excitation monochromator (wavelength calibration and intensity), measuring the water Raman (intensity), and a Starna standard of ovalene in polymethylmethacrylate (PMMA) for the emission monochromator (wavelength calibration and intensity). Wavelength accuracy was better than 1 nm and the water Raman was initially above manufacturer's specifications in intensity, indicating that the xenon lamp was more intense than expected. Excitation and emission spectra were measured using several excitation and detection wavelengths and several bandwidths (typically 5 or 10 nm), all of which were narrow compared to the broad emission and excitation spectra for the aromatic amines or their derivatives. Fluorescence references applicable to these types of samples are included for reference.[19,20] For the aromatic amines, monochromator bandwidths were 5.0 nm, 1 nm increments, and 0.1 second integration time. For the derivatized aromatic amines, monochromator bandwidths of 10 nm, 1 nm increments, and integration time of 0.1 second were used to lower the detection limit. Starna fused silica 1 cm path length cells were used with right-angle excitation for the solutions. For a few solutions, absorption spectra were recorded for comparison with the excitation spectra using a Spectral Instruments, Inc (Tucson, AZ) charge-coupled device (CCD) array UV-VIS spectrophotometer with fiber optics. ^{1}H nuclear magnetic resonance (NMR) spectra were measured on a Bruker (AMX300, $CDCl_3$) spectrometer at room temperature. Data were recorded as chemical shift values in ppm on the δ scale, multiplicity, integration, and coupling constants.

Reagents. The reagents were all used as received and were the highest grade obtainable. Only ethanol (200 proof USP or from Aldrich for microbiology) was used as solvent for the measurements reported here. Nitric acid (singly distilled from Aldrich) and HPLC grade water, also from Aldrich, were used for cell cleaning. Dinitrotoluene (2,4-DNT) was used from Sigma-Aldrich for the reduction reaction described below to produce diaminotoluene (2,4-DAT). 2,4-DAT was first obtained from Sigma-Aldrich (98%) and later from AccuStandard Inc (99.4%), 2,6-diaminotoluene (2,6-DAT) (99.7%) was also obtained from AccuStandard. Triaminotoluene (2,4,6-TAT) and aniline (aminobenzene) as likely typical minor components of an explosive vapor mixture after reduction were also obtained from AccuStandard Inc. O-Toluidine (97% or better) and p-toluidine (97% or better), also likely minor components of an explosive vapor mixture after reduction, were obtained from Sigma. A fluorescence derivatization agent fluorescamine (98%) was obtained from Molecular Probes, now a division of Invitrogen Inc. Although fluorescamine is supposed to be completely non-fluorescent in the absence of reactive amines, so that a surplus would not matter, it was found to be weakly fluorescent, presumably due to an unidentified impurity. Finally, fluorescein from Fluka (now affiliated with Sigma-Aldrich) was used to check the limit of detection of the instrument in a basic (NaOH) solution. For thin-layer chromatography (TLC) analysis, a Sigma-Aldrich TLC plate (silica gel with gypsum binder and fluorescent indicator) was used.

Synthesis of Catalyst. Catalytic nanoparticles of Pd were prepared in house for the reduction of nitro compounds such as DNT and other aromatic compounds as discussed in more detail in previous publications.[16–18] The water-in-hexane microemulsions were prepared at 25 °C by mixing 0.1 M of Na_2PdCl_4 solution (0.864 mL) with 200 mL of n-hexane and sodium dioctyl sulfosuccinate (AOT, 1.7782 g, 4 mmol), which gave a W value of 12;[18] W value is defined as the water to the surfactant molar ratio ([water]/[surfactant]). Multi-walled carbon nanotubes (MWCNTs) (1.0 g, 60–100 nm in diameter, Nanostructure & Amorphous Materials Inc, Los Alamos, NM) were pretreated by sonication in 14 M HNO_3 for 1 h and then refluxed for 12 h in a mixture of HNO_3 (50 mL, 14 M) and H_2SO_4 (98 % 50 mL). The treated MWCNTs (0.030 g) were added to the microemulsion solution with continuous stirring. Hydrogen gas at 1 atm was then bubbled through the solution for 30 min to reduce the Pd^{2+} ions dissolved in the water core of the microemulsion. It is known that hydrogen gas can cause reduction of Pd^{2+} ions in aqueous solutions to their elemental state.[17] After hydrogen reduction, the CNT-supported metal nanoparticles would precipitate to the bottom of the flask without stirring. The CNT-supported Pd nanoparticle catalyst was collected from the flask, washed with methanol, and dried in an oven for hydrogenation experiments.

Formation and Derivatization of DAT starting with 2,4-DNT. 2,4-Dinitrotoluene (0.0182 g, 0.1 mmol) in 10 mL of ethanol (10^{-2} M) with Pd/CNT (0.010 g) was stirred and then hydrogen was bubbled through the solution for 2 min at room temperature to make sure that DNT conversion to DAT was complete; TLC (eluent; 25% ethyl acetate and 75% n-hexane) was checked under UV light (254 nm) and then visualized by ninhydrin agent (red color). No starting materials were detected by TLC (DNT, R_f value = 0.45) and the product was DAT: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.83 (s, 1H), δ = 6.11 (d, 2H, J = 8.4 Hz), δ = 3.50 (s, 4H), δ = 2.08 (s, 3H).

To increase the fluorescence yield, DAT was reacted with fluorescamine reagent: 10^{-7} M DAT and 4×10^{-7} M fluorescamine (diluted down by a factor of 10 from the ethanol solution of 10^{-6} M DAT and 4×10^{-6} M fluorescamine ($4 \times$ 0.00278 mg), in 10 mL of ethanol and the solution was shaken for 2 min (longer than needed) at room temperature (RT). The solution was diluted by a factor of 10 after detection of fluorescence peaks and then this dilution procedure was repeated to reach 10^{-12} M of the solution. These DAT derivatives and other solutes being measured by fluorescence were stored in amber glass vials and further protected from ambient room light by aluminum foil to reduce risk of photoreactivity.

RESULTS AND DISCUSSION

A summary of the data for excitation and emission spectral peaks for the aromatic amines and their fluorescamine derivatives are given in Tables I and II. Figures 1–8 show the actual excitation and emission fluorescence data for the corrected and solvent-subtracted spectra: (Fig. 1) aniline, (Fig. 2) p-toluidine and o-toluidine, (Fig. 3) 2,6-DAT and 2,4-DAT, (Fig. 4) 2,4,6-TAT, (Fig. 5) aniline + fluorescamine, (Fig. 6) p-toluidine + fluorescamine and o-toluidine + fluorescamine, (Fig. 7) 2,4-DAT + fluorescamine at concentrations ranging from 10^{-8} M to 10^{-12} M, and (Fig. 8) 2,4,6-TAT +

TABLE I. Fluorescence data for aromatic amines.

Chemical	Excitation (nm)	Emission (nm)	Comments
Aniline	285	341	Ex & Em 10^{-6} M, bandwidths 5.0 nm
p-Toluidine	290	346	Ex & Em 10^{-6} M, bandwidths 5.0 nm
o-Toluidine	282	339	Ex & Em 10^{-6} M, bandwidths 5.0 nm
2,4-DAT (Diaminotoluene)	283	343	Ex & Em 10^{-6} M, bandwidths 5.0 nm
2,6-DAT (Diaminotoluene)	287	336	Ex & Em 10^{-5} M, bandwidths 5.0 nm
2,4,6- TAT (Triaminotoluene)	294	347	Ex & Em 10^{-4} M, bandwidths 5.0 nm

fluorescamine. The aromatic amines shown in Figs. 1–4 are likely fluorescent impurities in a mixture of explosive vapors after reduction, with 2,4-DAT being the major component. The aniline fluorescence spectrum was previously recorded in Ref. 9. The fluorescent derivatives of these compounds with fluorescamine[13] were also measured (Figs. 5–8) and in the case of 2,4-DAT as a function of several concentrations. DAT + fluorescamine led to a detection limit of 10^{-12} M when exciting at 392 nm (Fig. 7). The measured spectra, as are typical of aromatic phenols and amines, are broad (full-width at half-maximum (FWHM) of 56 nm for the emission spectra of 2,4-DAT 10^{-6} M) and without vibrational structure. They all have an excitation peak in the range of 280–300 nm and an emission peak between 330 and 350 nm. Although these spectra are similar, they can probably be distinguished by using synchronous luminescence and pattern recognition techniques, which we plan to discuss in follow-up publications. The excitation and emission spectra of the fluorescamine derivatives are red-shifted, broader (FWHM of 86 nm for the emission spectrum of 2,4-DAT at 10^{-9} M), and more intense. The emission intensity of a 10^{-6} M solution of DAT + fluorescamine derivative was found to be several orders of magnitude greater than a 10^{-6} M solution of DAT under the same experimental conditions (same bandwidths). The excitation peaks appear in a range of 380 to 410 nm. (For excitation purposes in a field instrument or sensor, it is preferable to excite as far towards the blue as possible, where better and cheaper sources exist.) The emission spectra of the fluorescamine derivatives show a single peak in the range of 480 to 510 nm. The instrumental conditions are given in each figure caption. For some compounds it was necessary to narrow the bandwidths to avoid entering the nonlinear saturation region for the photon-counting photomultiplier tube (PMT) detector, which was above 2.5 million counts per second (cps). All spectra were analyzed at least in duplicate; most were measured in triplicate or higher. Spectral peak heights for 2,4-DAT + fluorescamine were linear over the concentration range of 10^{-10} to 10^{-12} M, but were not linear above this concentration

TABLE II. Fluorescence data for derivatized aromatic amines.

Chemical	Excitation (nm)	Emission (nm)	Comments
Aniline + Fluorescamine	398	491	Ex & Em 10^{-8} M, bandwidths 5.0 nm
p-Toluidine + Fluorescamine	410	511	Ex & Em 10^{-6} M, bandwidths 5.0 nm
o-Toluidine + Fluorescamine	383	480	Ex & Em 10^{-6} M, bandwidths 5.0 nm
2,4-DAT + Fluorescamine	391	496	Ex & Em 10^{-8} M
		497	Ex & Em 10^{-9} M
		483	Ex & Em 10^{-10} M
		478	Ex & Em 10^{-11} M
		463	Ex & Em 10^{-12} M, detected 492 nm, bandwidths 10 nm
2,4,6-TAT + Fluorescamine	390	487	Ex & Em 10^{-6} M, detected 491 nm, bandwidths 10 nm

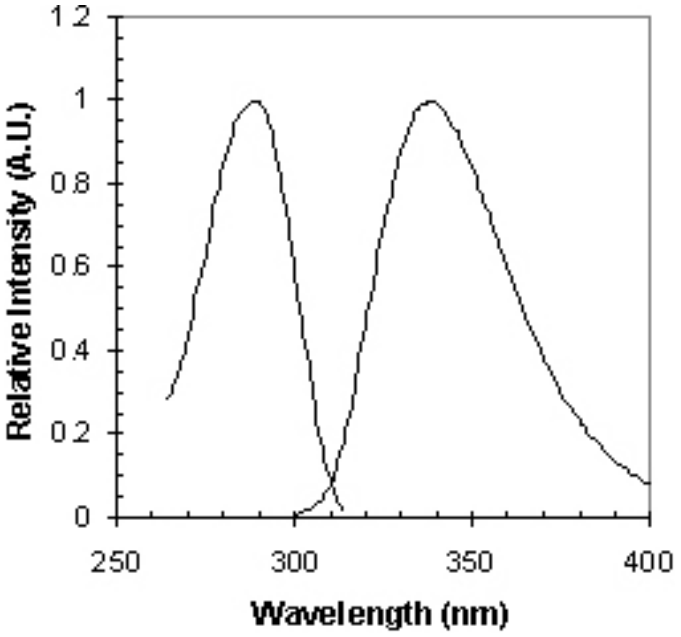

FIG. 1. Excitation and emission spectra of aniline 10^{-6} M. Bandwidth: 5 nm.

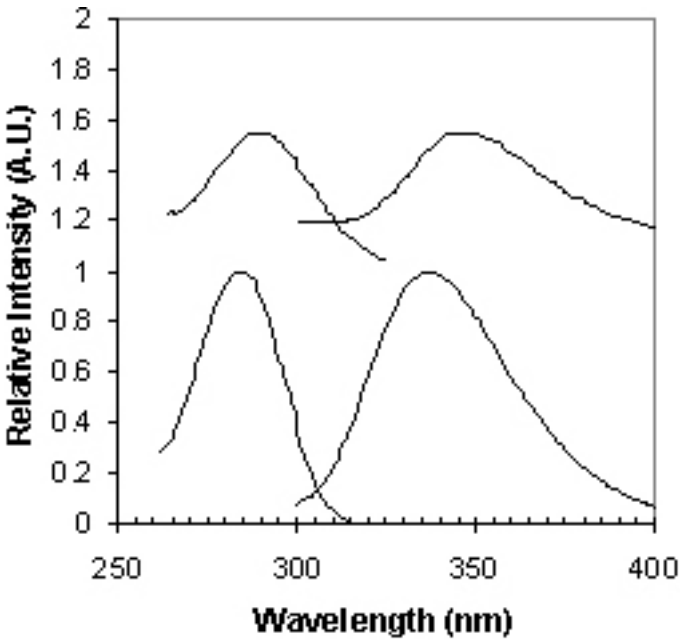

FIG. 2. Excitation and emission spectra of (***top***) p-toluidine 10^{-6} M and (***bottom***) o-Toluidine 10^{-6} M. Bandwidths: 5 nm.

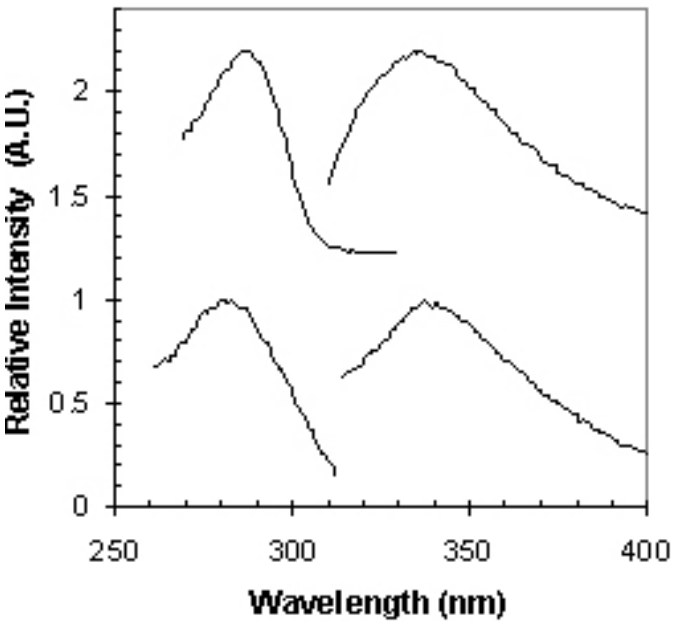

FIG. 3. Emission and excitation spectra of (***top***) 2,6-DAT 10^{-6} M and (***bottom***) 2,4-DAT 10^{-6} M. Bandwidths: 5 nm.

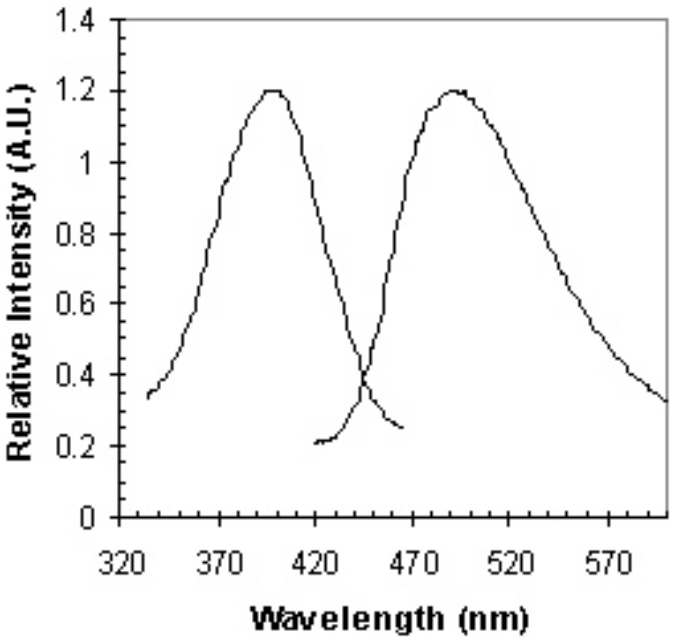

FIG. 5. Emission and excitation spectra of Aniline + Fluorescamine 10^{-8} M. Bandwidths: 10 nm.

even with solvent blank subtraction. In addition, a slight blue shift in the emission peak of 2,4-DAT after ethanol subtraction was observed as the concentration of DAT decreased probably due to a slight under-correction of the solvent blank on a very weak signal. 2,4-DAT was linear over the concentration range of 10^{-4} to 10^{-6} M. Provided the data are sufficiently reproducible, a calibration curve can be constructed even outside the linear range. For six replications of 2,4-DAT + fluorescamine at 10^{-6} M, the relative standard deviation was 11%. Most of the spectra are normalized to the highest fluorescence emission peak in the figure. Table I gives fluorescence spectral data for aromatic amines including excitation and emission peak positions, instrumental bandwidths, and concentrations (aniline, p-toluidine, o-toluidine, 2,4-DAT, 2,6-DAT, and 2,4,6-TAT). Table II gives fluorescence spectral data for derivatized aromatic amines including excitation and emission peak positions, instrumental bandwidths, and concentrations (fluorescamine derivatives of aniline, p-toluidine, o-toluidine, 2,4-DAT, 2,6-DAT, and 2,4,6-TAT).

Fluorescein standard (quantum yield approximately 0.9) in a basic solution was measured to give a detection limit of 10^{-12} M. This result was similar to what Spex had claimed for this

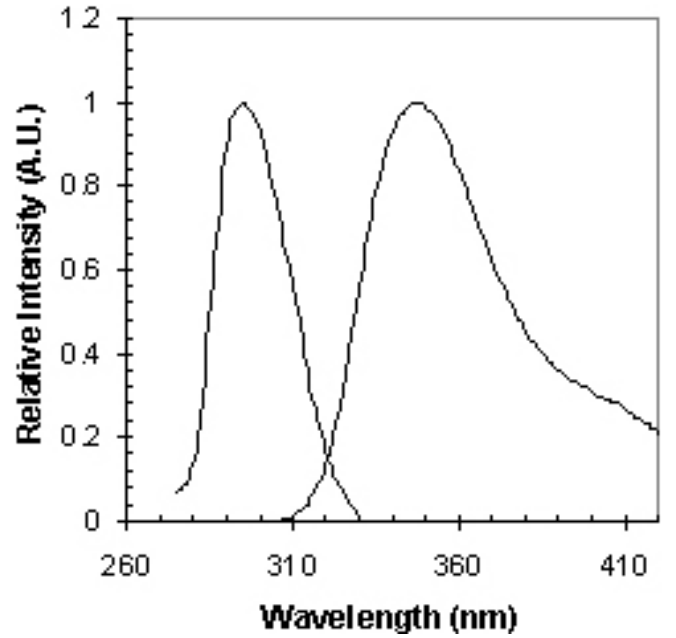

FIG. 4. Emission and excitation spectra of 2,4,6-TAT 10^{-4} M. Bandwidths: 5 nm.

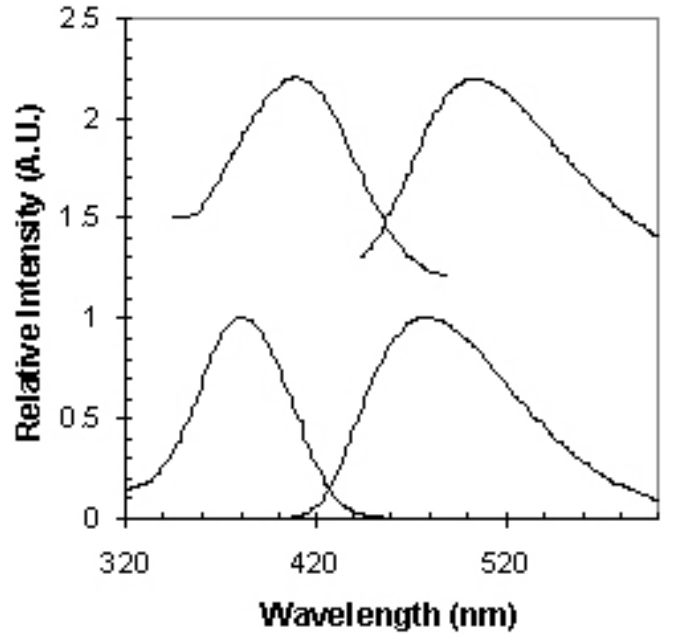

FIG. 6. Emission and excitation spectra of (***top***) p-Toluidine + Fluorescamine 10^{-6} M and (***bottom***) o-Toluidine + Fluorescamine 10^{-6} M. Bandwidths: 5 nm.

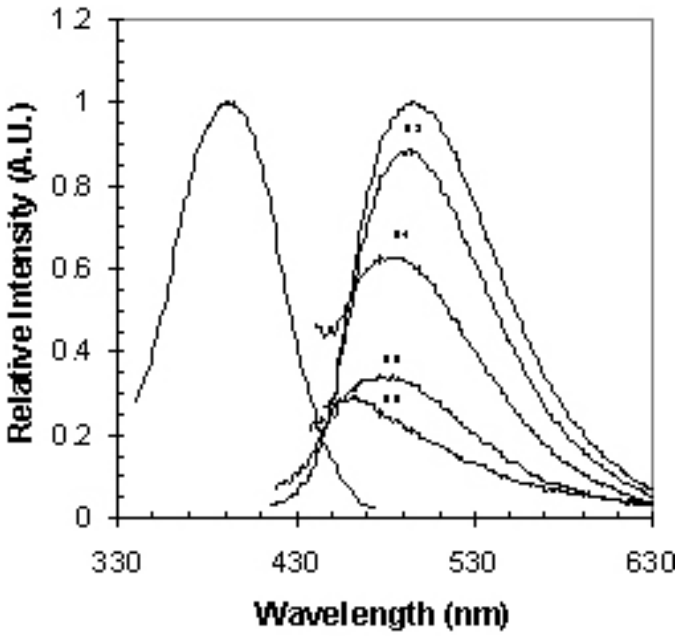

FIG. 7. Emission and excitation spectra of 2,4-DAT + Fluorescamine at 10^{-8} M, 10^{-9} M (multiplied by 3), 10^{-10} M (multiplied by 4), 10^{-11} M (multiplied by 5), and 10^{-12} M (multiplied by 5) in consecutive order (solvent blank subtracted). Bandwidths: 10 nm.

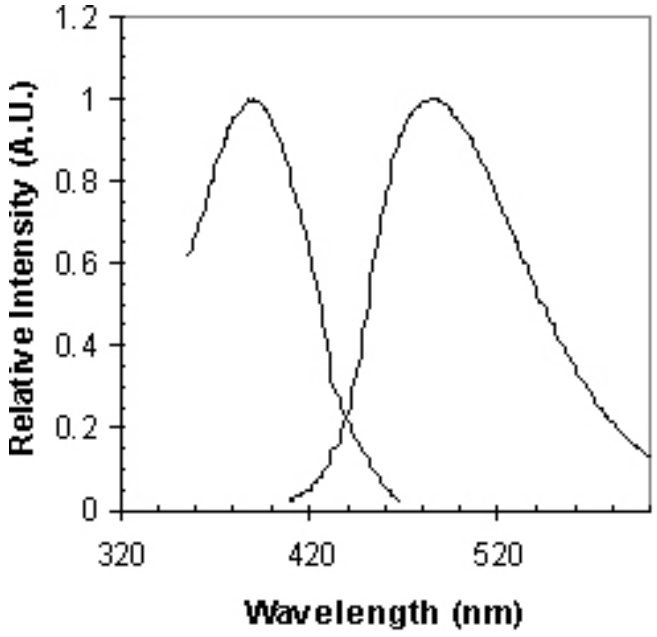

FIG. 8. Emission and excitation spectra of 2,4,6-TAT + Fluorescamine 10^{-6} M. Bandwidths: 10 nm.

instrument for fluorescein in a recent application note.[21] A FluoroLog-3™ instrument with a cooled PMT (which would further reduce instrumental noise and scattered light) can detect as low as 50 femtoMol of fluorescein.[21]

Recent experiments in our laboratory, using small stainless steel tubing with a diameter of 3 mm and a length of 13 cm packed with carbon nanotube-supported palladium nanoparticles for conversion of DNT to DAT in air with saturated DNT vapors and hydrogen gas (bubbling through an ethanol solution containing 10^{-6} M fluorescamine at 0.7 L per min at room temperature), showed that 1 min of flow was enough to detect the fluorescence emission of DAT–fluorescamine derivative in the solution.

CONCLUSION

The purpose of this preliminary paper was to show, using a commercial instrument, the feasibility of developing a fluorescence method or miniaturized field sensor for detection of explosive vapors as an alternative to fluorescence quenching methods currently being field tested. We have included a corrected updated UV-VIS fluorescence spectral library (Figs. 1–4) for the aromatic amines in Table I, and for their fluorescamine derivatives (Figs. 5–8), which have lower detection limits but broader spectra, in Table II. For 2,4-diaminotoluene + fluorescamine, we have measured the fluorescence intensity as a function of concentration, with a standard deviation of 11% at 10^{-6} M. The detection limit was found to be 10^{-12} M under the conditions of the experiment.

We also demonstrated the reduction of 2,4-DNT to the corresponding DAT and its reaction with fluorescamine. This was made by bubbling the DNT vapor through the Pd nanocatalysts, suspended on multiwalled carbon nanotubes, in the presence of hydrogen gas, and an ethanol solution of fluorescamine. Evidently, by using more powerful laser sources and more sensitive detectors with lower noise, the detection levels could be lowered further than the 10^{-12} M reported here, or the aromatic amines could be measured directly without need for derivatization, which would permit greater specificity, if needed. Reaction kinetics could be altered by changing experimental conditions to make the speed of the reactions more competitive with other procedures. The present conditions are optimized for 2,4-diaminotoluene reduced from 2,4-dinitrotoluene by a novel method. 2,4-DNT is likely to be the main component in explosive vapors leaking from a plastic landmine containing TNT.[1] An alternative approach might call for deposition on a solid substrate (cold finger) or to measure the DAT directly in the vapor phase. This method could be modified to apply to other explosive mixtures or be applied to explosives extracted from soil with supercritical fluid carbon dioxide.

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