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Coupling Paper-Based Microfluidics and Lab on a Chip Technologies for Confirmatory Analysis of Trinitro Aromatic Explosives

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ABSTRACT: A new microfluidic paper-based analytical device (μ PAD) in conjunction with confirmation by a lab on chip analysis was developed for detection of three trinitro aromatic explosives. Potassium hydroxide was deposited on the μ PADs (0.5 μ L, 1.5 M), creating a color change reaction when explosives are present, with detection limits of approximately 7.5 ± 1.0 ng for TNB, 12.5 ± 2.0 ng for TNT and 15.0 ± 2.0 ng for tetryl. For confirmatory analysis, positive μ PADs were sampled using a 5 mm hole-punch, followed by extraction of explosives from the punched chad in 30 s using 20 μ L borate/SDS buffer. The extractions had efficiencies of $96.5 \pm 1.7\%$. The extracted explosives were then analyzed with the Agilent 2100 Bioanalyzer lab on a chip device with minimum detectable amounts of 3.8 ± 0.1 ng for TNB, 7.0 ± 0.9 ng for TNT, and 4.7 ± 0.2 ng for tetryl. A simulated in-field scenario demonstrated the feasibility of coupling the μ PAD technique with the lab on a chip device to detect and identify 1 μ g of explosives distributed on a surface of 100 cm^2 .



The detection and analysis of explosives represents an important research field for forensic, environmental, and defense organization scientists. Rapid on-site methods are particularly valuable when it is necessary to analyze a large number of specimens. For example, rapid intelligence may be required following a terrorist incident by screening a large number of individuals or surfaces prior to confirmatory laboratory analysis.^{1,2} Similarly, inappropriate disposal of explosives that leads to soil contamination requires procedures to efficiently locate, identify and quantify the presence of environmental hazards.³

A wide range of analytical techniques have been developed for explosive detection including liquid chromatography mass spectrometry (LC-MS),⁴ high-performance liquid chromatography (HPLC),^{5,6} thin layer chromatography (TLC),⁷ capillary electrophoresis (CE),^{8–12} surface enhanced Raman spectroscopy (SERS),¹³ energy dispersive X-ray diffraction,¹⁴ ion mobility spectrometry (IMS),¹⁵ and gas chromatography mass spectrometry (GC-MS).^{16,17} However, while some of these techniques offer high sensitivity and selectivity,^{4–6,14,16,17} their long analysis times, relatively high costs and bulky instrumentation limit their use to laboratories and in the case of IMS, strategic locations, such as airports.

Capillary electrophoresis (CE) has been shown to be a reliable instrument for pre- and postblast explosive analysis^{18–20} and portable CE instruments have been developed. These can be either chip-based (lab on a chip) or capillary-based.^{21,22} Despite the increasing use of chip-based portable CE

in various fields^{23–25} capillary-based portable CE is also a viable approach.²²

Consequently, recent efforts have been focused on developing new, portable analytical approaches that enable rapid, cost-effective, on-site detection of explosives. Some of these techniques are based on amperometric detection and amperometric gas analysis,²⁶ polymer sensors,^{27,28} membrane-based flow displacement immunoassays,^{29–33} reflectance spectrometry,¹ and voltametric and fluorescence detection.^{34,35} Despite their applicability to in-field screening of explosives, these methods have limitations.^{1,36,37}

An extensively used method for the on-site analysis of explosives is colorimetric detection. Such techniques have a number of advantages including relatively high sensitivity and ease of use.^{38,39} Several research groups have focused on the development of colorimetric chemical sensors, which can be incorporated into portable screening systems.^{2,40} Such kits frequently rely on the Janowsky reaction which results in a red to violet color (Meisenheimer anion) when hydroxide or methoxide ions are mixed with trinitro aromatic compounds^{2,40,41} (Figure 1).

Variations of the Janowsky reaction have been used in spot tests, TLC plates,^{42–45} and more recently, in lab on a chip devices.² Lab on a chip devices are of particular interest for on-

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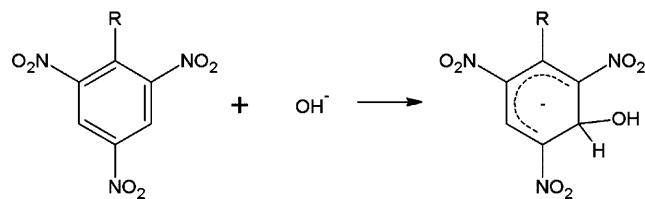


Figure 1. Reaction equation for the formation of the Meisenheimer anion (right side) from the reaction of trinitro aromatics with a base.

site detection of explosives because of their high sensitivity and selectivity, small sample size, and relatively low cost.^{2,46–48}

A new generation of microfluidics, the microfluidic paper-based analytical devices (μ PADs), were introduced in 2007.⁴⁹ Paper-based microfluidic devices have recently been extensively reviewed by Yetisen et al. (2013) covering the current capabilities and limitations of the performance of such devices.⁵⁰ Important advantages of μ PADs over other microfluidic devices which are applicable to development of portable devices for explosives detection include low cost, easy storage and disposal, and no requirement for pumps. μ PADs have been applied to colorimetric assays for a number of analytes in health diagnostics including glucose, protein, ketones, and nitrates.^{49,51}

The fabrication of μ PADs involves the construction of patterns of hydrophobic barriers on filter paper, using techniques such as photolithography,⁵² plotting,⁵³ paper cutting⁵⁴ plasma oxidation,⁵⁵ inkjet printing,⁵⁶ inkjet etching,^{57,58} flexographic printing,⁵⁹ laser treatment,⁶⁰ or wax printing.^{61,62} The hydrophobic barriers allow controlled fluid movement that segregates chemical reactions. Not only are the μ PADs ideal for in-field applications but also their simple fabrication allows production in less sophisticated laboratories. We have recently reported the application of a portable μ PAD device based on fluorescence quenching of pyrene for the detection of organic explosives following sample collection from surfaces using a swab. Solvents effects were also evaluated.⁶² Here, we have expanded this technology to develop a μ PAD-based colorimetric assay for the detection of three related trinitro aromatic explosives which can be combined with lab on a chip technology. Following the μ PAD returning a positive result for these compounds, the sample is able to be further analyzed using a lab on a chip device to confirm the presence of explosive and determine the identity of the compound.

MATERIALS AND METHODS

Chemicals. All reagents and chemicals were analytical grade. Sodium dodecyl sulfate (SDS) was purchased from ChemService (West Chester, PA, U.S.A.). Certified explosive solutions (1000 μ g/mL) in acetonitrile comprising 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), 4-amino-2,6-dinitrotoluene (4-A-2,6-DNT), 4-nitrotoluene (4-NT), 1,3,5-trinitro-1,3,5-triazaacyclohexane (RDX), 2,4,6-trinitrophenylmethylnitramine (tetryl), 2,4-dinitrotoluene (2,4-DNT), 2-nitrotoluene (2-NT), and 2,6-dinitrotoluene (2,6-DNT) were also purchased from ChemService (West Chester, PA, U.S.A.). Certified solutions (100 μ g/mL) of triacetone-triperoxide (TATP), ethylene glycol dinitrate (EGDN), octahydrotetranitrotetrazine (HMX), pentaerythritol tetranitrate (PETN), 3,4-dinitrotoluene (3,4-DNT) were obtained from AccuStandard (New Haven, CT, U.S.A.). Ultrapure water ($18.2\text{ M}\Omega\text{ cm}^{-1}$) was used throughout all experiments (Sartorius 611 water purification system). Acetonitrile, sodium

chloride (NaCl), and sodium tetraborate decahydrate (borate buffer) were obtained from Sigma-Aldrich (Australia). Methanol was obtained from Chem-Supply Pty Ltd. (Australia). DNA dye (blue) was obtained from Agilent Technologies, (Lithuania). Sodium hydroxide (NaOH) was purchased from Univar (Ajax Finechem, Australia). Potassium hydroxide (KOH) was obtained from Biolab (Australia) and 36% hydrochloric acid (HCl) solution was obtained from Labscan (Australia). Ammonium nitrate (AN) was obtained from BDH (VMR International).

All explosives were stored in sealed glass containers in an explosion proof freezer (-20°C). All experiments were conducted in well ventilated fume hoods with appropriate personal protection, that is, face protection, gloves, and lab coats.

Black tea (Lipton), Coca Cola, white wine (Sauvignon Blanc Semillon), full cream milk (Coles), orange juice (Berri), perfume (Le Bouquet Absolut Givenchy, Miss Dior Blooming Bouquet), cleaning product (Ajax Spray n' Wipe Multipurpose), surfactant (Daily Shower Cleaner, Method), oxidizing agents (Vanish Oxi Action Fabric Stain Remover and Dylon Stain Remover), washing powder (OMO Sensitive), deodorant (Nivea) were purchased at Australian supermarkets and diluted 1:4 immediately before use.

Instruments. CorelDraw 5 software (Corel Corporation, Ontario, Canada) was used to design the μ PADs. A Fuji Xerox ColorQube 8870 printer (Xerox, Australia) was used to print the μ PADs. Whatman 185 mm diameter filter paper grade 41:20 μm (Ashless grade, Ash 0.01%, England) was used. The printed wax was melted using a swing-away heat press (GEO Knight & Co, Inc.) to obtain the μ PAD.

A Video Spectral Comparator VSC6000 (Forster+Freeman Ltd.-England) was used to digitally capture and analyze the color reactions. Color development was evaluated after 10 min reaction time. A blank area of the μ PAD was used as the background reference for all colorimetric measurements. The experiments were repeated three times and the data compared using a Student's *t* test, a *p* < 0.05 was considered significant.

Explosives were separated and identified on DNA chips using an Agilent 2100 Bioanalyzer controlled by Agilent 2100 Expert software (Agilent Technologies, Waldbronn, Germany).

μ PAD Fabrication. μ PAD designs with circles of diameters ranging from 5 to 10 mm and hydrophobic borders with a line width of 0.5 mm were drawn and tested for sampling applications. The devices were printed on filter paper and heated at 150°C for 5 min. For the 5 mm and 8 mm, μ PADs, 0.5 and 1.3 μL of either KOH or NaOH (1.5 M) were deposited, respectively.

KOH and NaOH solutions were investigated as colorimetric reagents to detect TNT. KOH and NaOH solutions ranging from 12.5 mM to 2 M were prepared by dilution of a 2 M stock solution in water. 0.5 μL of each dilution were deposited on a μ PAD and allowed to dry for 2 min. The TNT standard (1000 ppm) was diluted 1:1 in methanol/water (80:20) and 0.25 μg of TNT (0.5 μL , 500 ppm) was pipetted on to each μ PAD. Following a 10 min reaction time, the color intensity was measured using the Video Spectral Comparator VSC6000 at 480 nm.

Collection Techniques. A 10 cm \times 10 cm bench surface was used to test two different collection techniques. After the bench surface was cleaned with methanol, the absence of explosive material was confirmed by testing the surface with a prepared μ PAD. After a negative result, 10 μg TNT, TNB, and

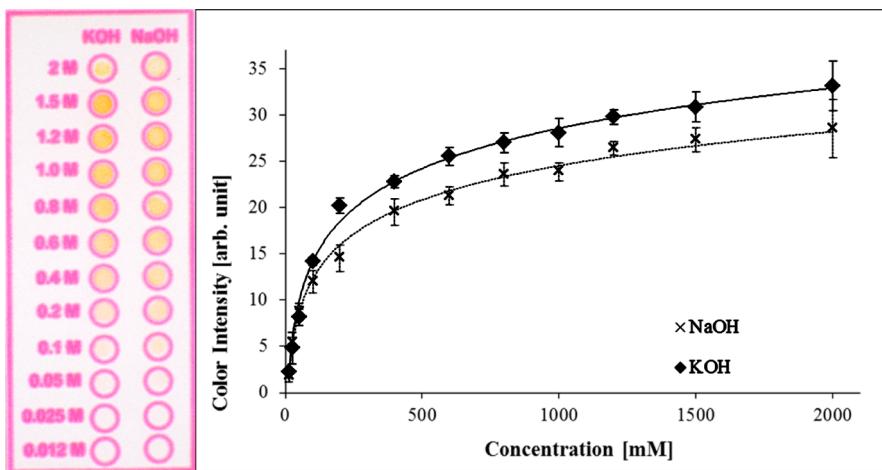


Figure 2. Picture of the color formed on the μ PAD after reaction between $0.5 \mu\text{L}$ TNT (500 ppm) in methanol:water and the bases KOH and NaOH ($0.5 \mu\text{L}$ previously deposited on the μ PAD) (left). Plot of the color intensity formed on the μ PAD versus concentration of the two bases (right). Color intensity was determined ($n = 3$) with a video spectral comparator VSC6000 measuring pixel density, in arbitrary units). Error bars are SD of the mean.

tetryl solutions (each $10 \mu\text{L}$, 1000 ppm) were spiked homogeneously on to the surface and allowed to dry.

The first technique was sampling the surface with a cotton swab dampened with methanol/water (80:20) and transferring the sample to the μ PAD by pressing the swab onto the surface for 1 s in the active circle.

The second technique consisted of direct collection by moistening the surface with $100 \mu\text{L}$ of methanol/water (80:20) and sampling the surface with the active portion of the μ PAD.

Lab on a Chip. All experiments were performed on an Agilent 2100 Bioanalyzer. Indirect detection was performed using laser emitting diode-induced fluorescence ($\lambda_{\text{ex}} = 635 \text{ nm}$, $\lambda_{\text{det}} = 680 \text{ nm}$). Separations were performed on standard DNA 500 microchips obtained from Agilent Technologies (Forest Hill, Australia). The glass chips have 12 sample wells and microchannels with a depth of $10 \mu\text{m}$, a width of $50 \mu\text{m}$ and a separation channel length of 15 mm. The samples were injected using pinched mode at 1500 V for 40 s and separated using 1500 V for 100 s.

Electrolyte and Sample Preparation for the Bioanalyzer. Solutions of 10 mM borate buffer containing 50 mM SDS were prepared and filtered using a $0.20 \mu\text{m}$ nonpyrogenic single use syringe filter (Minisart Syringe Filters, Sartorius). The solutions were then sonicated for 5 min prior to addition of 1.5% (v/v) of the Agilent Bioanalyzer DNA dye for indirect detection of the explosives by fluorescence quenching.

Explosive standard solutions (200 ppm to 0.625 ppm) were prepared by dilution in 10 mM borate buffer containing 50 mM SDS.

Collection and Identification. TNT, TNB, and tetryl ($2 \mu\text{g}$; $10 \mu\text{L}$ of a 200 ppm solution) were applied to a clean $10 \text{ cm} \times 10 \text{ cm}$ bench surface and collected using the direct collection technique. One active μ PAD with two spots, one containing $1.3 \mu\text{L}$ KOH 1.5 M (active spot) and an inactive spot (no reagent on the spot) was used to sample the surface. The active spot was used to verify the color reaction while the inactive spot was removed using a 5 mm hole-punch for extraction of explosives. This filter paper punch was placed into a microfuge tube (0.6 mL) and explosives were extracted by addition $20 \mu\text{L}$ of 50 mM SDS/10 mM borate solution followed by stirring for 30

s. Nine microliter samples of the extract were then analyzed in triplicate using the Agilent 2100 Bioanalyzer.

RESULTS AND DISCUSSION

Using a base to detect nitro aromatics is a simple chemical procedure and is highly specific for trinitro aromatic explosives, as the analyte is part of product molecule.⁶³ Dinitro aromatics have also been shown to produce positive color reactions, but the reaction times exceed the practical time frame for on-site explosive detection.⁶⁴ Two strong bases (KOH and NaOH), were evaluated as potential reagents for the development of a microfluidic paper-based analytical device based on their previously reported use.⁶⁴

There were significant differences ($p < 0.05$) in the color intensity between KOH or NaOH at base concentrations above 400 mM when reacted with TNT (Figure 2). This was likely a result of the greater solubility of KOH than NaOH in 80% methanol, which was used to dilute the TNT standard. In this experiment the bases were tested against a higher concentration of TNT than would be likely to be encountered in field-use situations to ensure that color development would not be limited by the availability of base. Color development following exposure of the base to trinitro explosives was observed immediately (<30s), but was measured after 10 min to ensure completion of the reaction. This short reaction time fulfills the requirements for rapid detection when developing a portable infield method. Color was observed to remain stable for greater than one month. Results for TNB and tetryl were similar (data not shown). A concentration of 1.5 M KOH was chosen for all further experiments as above this concentration there is little advantage in terms of increased color development (Figure 2).

Limit of Detection of the μ PAD. The limits of detection (LODs) of TNT, TNB and tetryl on the μ PAD were determined after application of $0.5 \mu\text{L}$ of each explosive at concentrations ranging from 1 to 200 ppm onto the active device using a video spectral comparator VSC6000 at 480 nm (Figure 3).

The minimum visually detectable levels on the μ PAD were $30 \pm 3 \text{ ng}$ for TNB, $50 \pm 4 \text{ ng}$ for TNT, and $70 \pm 2 \text{ ng}$ for tetryl. The minimum detectable amounts (MDAs) using the VSC6000 were $7.5 \pm 1.0 \text{ ng}$ for TNB, $12.5 \pm 2.0 \text{ ng}$ for TNT,

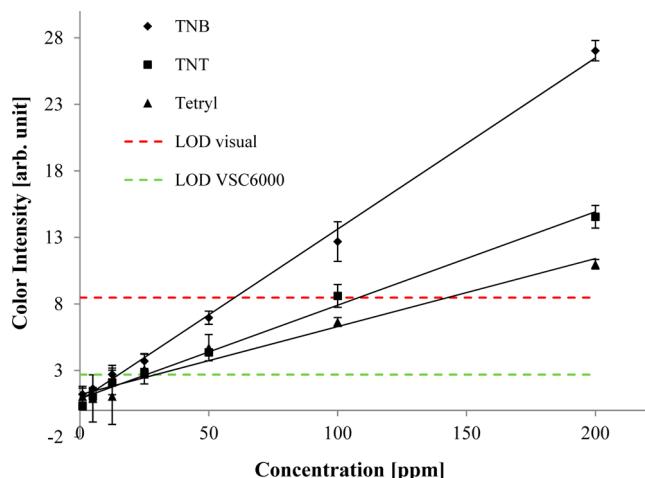


Figure 3. Color intensity formed on the μ PAD measured at 480 nm using a VSC6000 spectral comparator following the reaction of 0.5 μ L of 1, 5, 12.5, 25, 50, 100, and 200 ppm of TNT, TNB, and tetryl with 0.5 μ L of 1.5 M KOH solution (previously deposited) on a μ PAD ($n = 3$).

and 15.0 ± 2.0 ng for tetryl. These MDAs are comparable to those obtained using currently available commercial colorimetric tests.³⁸

Interference. False positive results may occur in the presence of related structures to those of the explosives to be detected; such structures include an aromatic moiety with a minimum of two nitrate substitutions. For example perfumes may contain nitro aromatic compounds (nitro musks), which have been reported to interfere with colorimetric field tests for explosives.⁶⁵ The selectivity of the μ PAD was evaluated by analyzing 14 common substances which were chosen on the basis of their potential to interfere with color detection or to affect the availability of the base. These compounds include cleaners, soap, deodorant, wine, detergents, tea, cola, milk, orange juice, salt, and different explosives. These substances (excluding the explosives), represent materials which may be found in ambient environments and may be a source of interference. At the concentrations tested, no color develop-

ment was observed when reacted with KOH on the μ PAD (Figure 4A) indicating significant selectivity of the proposed detection method. However, there is potential for interference with the colorimetric reaction either from the presence of inorganic acids which may give false negatives; or nitro aromatics which may give a false positive. False positives would be detected by subsequent analysis using the lab on a chip.

Several types of explosives were examined to determine the selectivity of the μ PAD (Figure 4B): 0.1 μ g of 2,4-DNT, 3,4-DNT, 2,6-DNT, 4-NT, 2-NT, EGDN, PETN, 4-A-2,6-DNT, TNT, TNB, tetryl, HMX, RDX, TATP, and AN (0.5 μ L, 200 ppm) were added onto the μ PAD. The resulting color reactions were monitored by measuring the color intensity at 480 nm using a VSC6000 spectral comparator. Only TNT, TNB, and tetryl, which differ only in one substitution on the benzene ring (Figure 4 C) resulted in a positive color reaction. The DNT isomers were able to produce positive results, however, these reactions took up to 2 days to form a colored product on the μ PAD and so would not interfere with detection of trinitro aromatic explosives, which react very rapidly (within seconds).

Collection Techniques. The direct collection technique for the μ PAD (Figure 5 B) was more efficient than using a swab

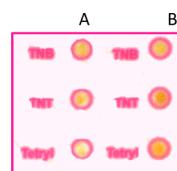


Figure 5. Ten micrograms of each explosive was homogeneously distributed onto individual surfaces of 10 cm \times 10 cm and collected using either (A) a cotton swab dampened with methanol–water solution (80:20) or (B) the μ PAD was used to collect the sample directly, following application of 100 μ L of the same solution to the surface.

(Figure 5 A). This is likely due to lower efficiency of transfer of the explosives from the cotton swab to the paper surface. Using the μ PADs to directly sample the surface increased sample

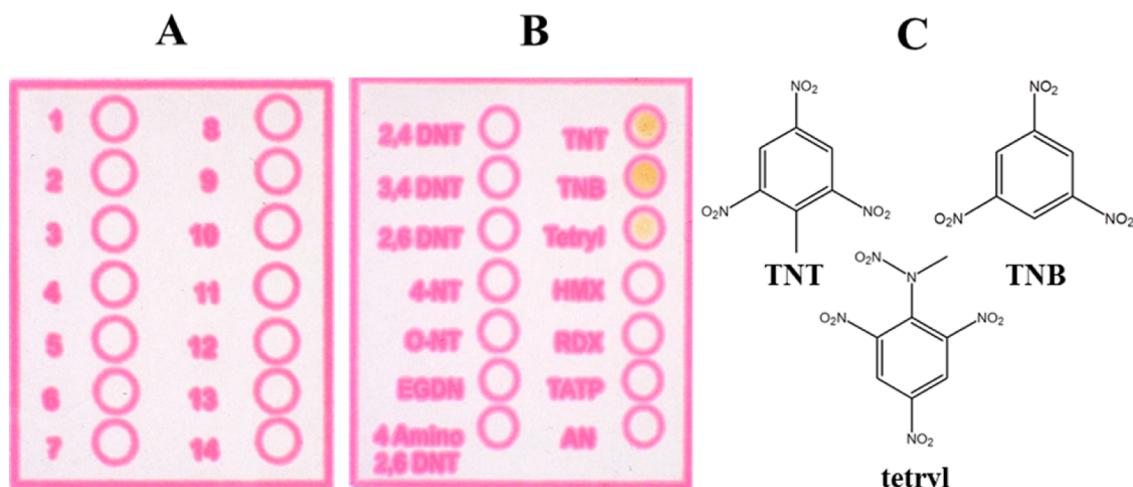


Figure 4. μ PAD with 0.5 μ L of 1.5 M KOH deposited reacting with 0.1 μ g (0.5 μ L 200 ppm) of (A) 1 perfume, Givenchy; 2 perfume, Dior; 3 cleaner; 4 surfactant; 5 deodorant; 6 white wine; 7 oxidizing agent, Vanish; 8 oxidizing agent, Dylon; 9 washing powder; 10 black tea; 11 cola; 12 milk; 13 orange juice; 14 NaCl (1000 ppm) and (B) (explosives, 0.5 μ L, 200 ppm) 2,4-DNT, 3,4-DNT, 2,6-DNT, 4-NT, O-NT, EGDN, PETN, 4-A-2,6-DNT, TNT, TNB, tetryl, HMX, RDX, TATP, and AN. (C) Chemical structure of the explosives producing a positive detection reaction.

recovery and reduced sampling time. For this reason, the direct collection technique was used in all further experiments.

Optimization of the μ PAD Design. Our results indicate that the size of the circle where the reagent is deposited should be as small as possible to maximize the intensity of the color reaction. Further, limitations to the maximum size of the device must be considered to avoid nonreactive regions which may decrease the sensitivity of the device.

A large hydrophobic border ($15\text{ mm} \times 20\text{ mm}$) was used to define the reaction area and minimize the available nonreactive surface area. The optimized reaction area was printed as an 8 mm diameter circle which results in a 6 mm diameter test area on the fabricated μ PAD after heating. This diameter is sufficiently small to concentrate the color reaction for clear visualization. In addition, this size facilitates removal of a disc (using a 5 mm diameter hole punch) without contamination of the chad by the ink for further lab on a chip analysis. $1.3\text{ }\mu\text{L}$ of the 1.5 M KOH solution was required to fill the test area. The lower area of the μ PAD was left unprinted for labeling and handling.

When the active surface of the μ PAD containing KOH was used to extract the collected explosive residues the electropherograms obtained were of poor quality and the explosives could not be separated. This was likely because of the elevated pH overwhelming the buffering capacity of the electrolyte. For this reason, μ PADs with two circles were designed, one containing KOH and one without the reagent.

To collect samples only once and ensure that they were identical, both circles were simultaneously brought into contact with the surface to be tested. When a positive color reaction occurred on the KOH circle (color), the circle without the KOH reagent (punch) was hole-punched and used for extraction (Figure 6).

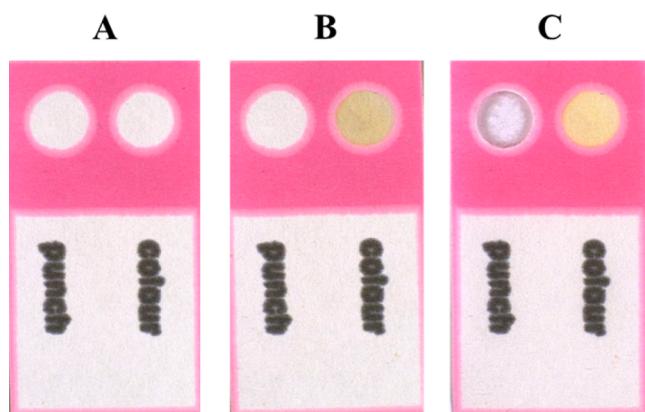


Figure 6. (A) Design of the optimized μ PAD for the on-site collection of explosives. (B) μ PAD after sampling a bench surface of $10\text{ cm} \times 10\text{ cm}$ spiked with $5\text{ }\mu\text{g}$ of TNB ($10\text{ }\mu\text{L}, 500\text{ ppm}$). (C) μ PAD showing the left spot (that does not contain KOH) punched for lab on a chip analysis.

Lab on a Chip Separation. The most successful strategy used to analyze organic explosives in CE is by micellar electrokinetic chromatography with borate buffer containing SDS as the electrolyte.⁶⁶ Explosives do not fluoresce without some form of derivatization. However, explosives are well-known to quench fluorescence.⁶⁷ Fluorescence quenching as a method of detection for explosives has been applied to CE, HPLC and more recently, lab on a chip devices.⁶² The

Bioanalyzer 2100 is a commercial lab on a chip system fitted with both LED-IF and LIF detection systems. The device is compact and portable, with a mass of $\sim 10\text{ kg}$ allowing onsite operation. This instrument has been reported to have application to in-field analysis of illicit drugs^{23,68} and DNA analysis.^{24,25}

Detection of explosives extracted from the μ PAD was performed using an indirect fluorescence assay in which the explosive molecules quench the fluorescent dye (DNA dye) present in the buffer, producing a negative peak. The separation of TNB, TNT, and tetryl on this device was optimized by varying parameters such as pH, borate buffer concentration, DNA dye concentration, and the separation voltage. The addition of 50 mM SDS to the borate buffer was necessary to create a pseudo stationary phase that permits the separation of the neutral nitroaromatic compounds.⁹

Borate buffer solutions (5 to 25 mM) with pHs of 9.00, 9.20, and 9.40 containing 50 mM SDS were tested. A borate buffer concentration of 10 mM was chosen as this gave improved separation and reduced baseline noise compared to higher concentrations. A pH of 9.2 was determined to be optimal for this separation. Buffers below this pH gave poor separation, while buffers with pH 9.4 caused precipitation of SDS.

The DNA dye was mixed in the separation buffer to permit indirect detection. A range of dye concentrations from 0.25% to 1.5% were evaluated. The greatest sensitivity was obtained using 1.5% and this concentration was used for all further experiments as decreasing the concentration to 1% halved the peak intensity. Higher concentrations of the dye may increase the sensitivity however; additional costs and potential saturation of the detector outweigh any advantage.

A standard mixture of the three target explosives was either directly analyzed or sampled from the test surface with the μ PAD, extracted and then analyzed using the Agilent Bioanalyzer. The extraction procedure was carried out using the same buffer as that for the separation and the optimal extraction of explosives was obtained by stirring the paper punch in the solution as described above. Recovery of sample from the punch was $96.5 \pm 1.7\%$ (data not shown) while recovery from the surface was approximately 12% of the deposited explosive sample (Figure 7). This recovery may potentially be further optimized to improve efficiency by using alternative solvents commonly used for extraction of explosives such as acetonitrile or acetone.

Figure 7 shows successful separation of the three explosives from the μ PAD compared to the standard mixture. It can be seen that the use of the μ PAD not only allows the rapid detection of trinitro aromatic explosives but also the subsequent identification of these species and elimination of any false positive results that may be obtained.

The minimum detectable amounts of the explosive using the Agilent Bioanalyzer were $7.0 \pm 0.9\text{ ng}$ for TNT, $3.8 \pm 0.1\text{ ng}$ for TNB, and $4.7 \pm 0.2\text{ ng}$ for tetryl (based on a 3:1 signal-to-noise ratio) and, as expected, were lower than those obtained for the color reaction either visually or using the VSC6000 spectral comparator. Figure 8 illustrates the simple steps involved in trinitro aromatic explosive analysis using the method described above, from sample collection and colorimetric detection to identification.

CONCLUSIONS

A new microfluidic paper-based analytical device was developed to detect trinitro aromatic explosives at on-site scenarios. KOH

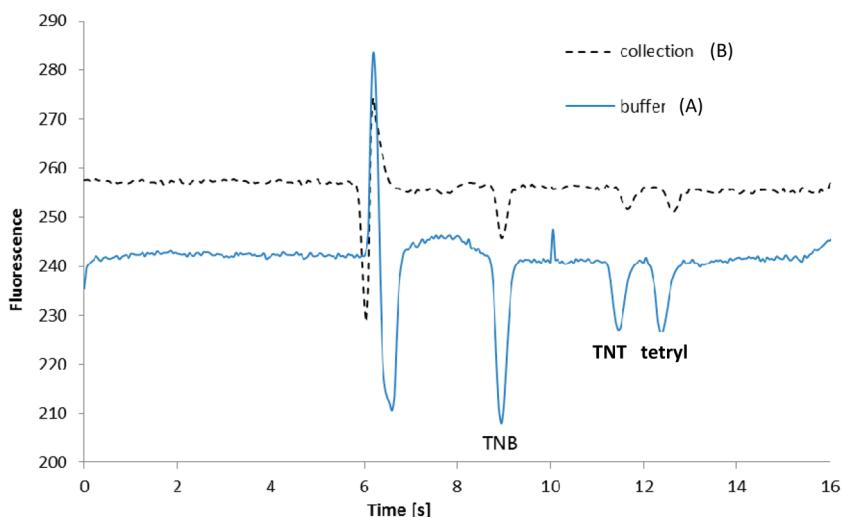


Figure 7. (A) Electropherogram of the standard mixture of 0.3 μg each of TNT, TNB, and tetryl (3 μL , 100 ppm) in 17 μL 50 mM SDS/10 mM borate buffer. (B) Electropherogram of the extractant from the μPAD after sampling of a standard mixture containing 1 μg of TNT, TNB, and tetryl (10 μL , 100 ppm in methanol:water) spiked onto a clean 10 cm \times 10 cm surface. All experiments performed using the 2100 Bioanalyzer (injection 1500 V/40 s, separation 1500 V).

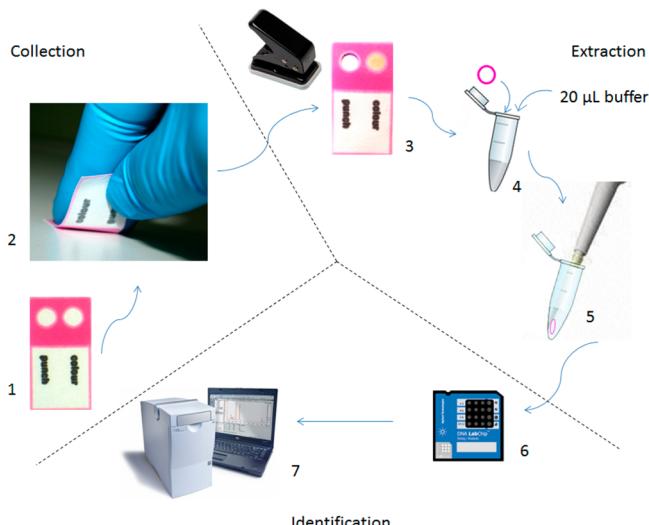


Figure 8. (1) μPAD , (2) direct collection after spraying the surface with a methanol/water (80:20) solution, (3) inactive spot is punched out using a hole-punch, (4–5) the chad is moistened with 20 μL of the electrolyte and extracted by mixing with the pipet for 30 s, (6) 9 μL of the extracted sample is added on the microchip, and (7) analysis using the lab on a chip 2100 Bioanalyzer.

(1.5 M) was used as a reagent on the μPAD to form the active spot test. The limit of detection of the μPAD was 7.5 ± 1.0 ng for TNB, 12.5 ± 2.0 ng for TNT, and 15.0 ± 2.0 ng for tetryl when using a spectral comparator. Concentrations higher than 30 ng could be detected by visual inspection of the device. No loss in sensitivity was observed using one month old μPAD s, suggesting that storage for field-based use is promising. The optimized μPAD design consists of two circles, one used for the color reaction (color), and one for later extraction and lab on a chip analysis (punch). The extraction of the collected explosives in the 5 mm diameter chad was achieved using 20 μL of 50 mM SDS/10 mM borate buffer pH 9.2 with an efficiency of $96.5 \pm 1.7\%$. Separation and identification of the explosives were achieved using an Agilent Bioanalyzer lab on a chip instrument with indirect fluorescence detection. The

separation was performed at 1500 V using the same buffer used during the extraction containing 1.5% DNA dye. The minimum detectable amounts were 7.0 ± 0.9 ng for TNT, 3.8 ± 0.1 ng for TNB and 4.7 ± 0.2 ng for tetryl. In a simulated on-site scenario the color reaction tested positive for 1 μg of the three explosives distributed on a surface of 100 cm². Future experiments may be focused on the optimization of solvent composition. While detection was adequate for the purpose, surface recovery could be improved to expand application of the method in the field. Although we did not perceive problems with our μPAD s, consideration should be taken during further development of μPAD devices based on color reactions to avoid possible ink contamination.

This research demonstrates a low cost and portable μPAD for the detection of trinitro aromatic explosives. Combining this procedure with a lab on a chip device permits a confirmatory identification of the collected explosives. The overall procedure is quick and field portable.

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Notes

The authors declare no competing financial interest.

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