

Detection of trinitrotoluene (TNT) extracted from soil using a surface plasmon resonance (SPR)-based sensor platform

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ABSTRACT

An antibody-based competition assay has been developed using a surface plasmon resonance (SPR) sensor platform for the detection of trinitrotoluene (TNT) in soil extract solutions. The objective of this work is to develop a sensor-based assay technology to use in the field for real-time detection of land mines. This immunoassay combines very simple bio-film attachment procedures and a low-cost SPR sensor design to detect TNT in soil extracts. The active bio-surface is a coating of bovine serum albumin that has been decorated with trinitrobenzene groups (TNB-BSA). A blind study on extracts from a large soil matrix was recently performed and results from this study will be presented. These will include demonstrated sensitivity to 1ppm TNT, in a variety of soils, with no false negatives. Potential interferants studied included 2,4-dinitrophenol, 2,4-dinitrotoluene, ammonium nitrate, and 2,4-dichlorophenoxyacetic acid. Cross-reactivity with dinitrotoluene will be discussed. Also, plans to reach sensitivity levels of 1ppb TNT in soil will be described.

Keywords: biosensor, land mines, TNT, trinitrotoluene, SPR, surface plasmon resonance

1. INTRODUCTION

Today, there is a great deal of research aimed at the detection of unexploded ordnance, especially land mines. These efforts result, in part, from the great numbers of civilians placed in peril by such devices. Land mines buried during times of war are often not removed or detonated and can pose a serious risk to a population trying to rebuild in times of peace.^{1,2}

There are several methods currently used to detect buried land mines. Mechanical methods of searching for and clearing land mines are time and labor intensive. Metal detectors are widely used, but are becoming less effective as more and more mines are made from plastics and alternate materials. Metal detectors are also subject to metallic interferants. Canines are extremely effective at finding buried land mines, but they require extensive training and tire easily.

For these reasons, new techniques are being developed for sensing buried land mines. The present paper describes one such activity. We present here an immunoassay platform used to transduce the binding of an antibody into an electronic signal for the detection of 2,4,6-trinitrotoluene (TNT) a common explosive used in the manufacture of land mines. The work is based on the principle of surface plasmon resonance (SPR). SPR has been described in numerous reviews.^{3,4} Simply put, SPR allows for the monitoring of refractive index changes localized within a few hundred nanometers of an active gold surface. The surface-sensitive nature of SPR lends itself to kinetic studies of molecular binding events. SPR has been used for many years as a signal transduction method to monitor biochemical interactions in real-time.⁵ As a result, commercial instruments have been developed to study a wide variety of bio-molecular binding events under controlled laboratory conditions.^{6,7}

Given the rich history for SPR in laboratory based bio-sensing work, it seems a natural candidate method for a more *distributed* testing scheme. That is, one where active sensors are deployed in remote locations rather than having all measurements performed in a centralized testing location. Some of the many advantages that distributed sensing offers include the elimination of sample transportation, parallel test capability, and more timely access to the test results.

Several issues must be addressed for such a methodology to be useful in an uncontrolled field environment. The SPR transducer must be rugged, fully integrated, and miniature. Given that the SPR transducer requires a liquid sample, a method must be devised for collecting the TNT around a land mine and transferring it to a liquid medium. It is observed that explosive particulates partition more into soil than water and air, and some eventually form a crust near the surface of the ground.⁸ One obvious avenue is to extract the TNT from the soil surrounding a mine using a solvent. Ideally the SPR transducer would be capable of handling liquid samples containing potential interferants and contaminants.

Texas Instruments Incorporated has developed an SPR transducer, Spreeta™, which meets several of these requirements. To demonstrate the feasibility of using Spreeta to detect TNT in soil samples, a double blind study was conducted by the Defense Advanced Research Projects Agency (DARPA) in Dallas on August 17-18, 1998. The results of this study are presented here.

2. EXPERIMENTAL

2.1 The Spreeta™ sensor

The Spreeta™ sensor has been described in detail previously.^{9, 10} Briefly, all optical and electronic components required for SPR are die mounted and wire bonded onto a small (400mm²) platform using normal electro-optical device manufacturing techniques. The devices are then encapsulated in an optical material using a cast mold process. This material provides all necessary optical surfaces as well as protection for the electronic components.

During operation, light from an 830nm AlGaAs LED (Infratech Incorporated, Garland, TX) is passed through an aperture in the light source housing and is polarized. The light is then directed through the molded epoxy (Ciba-Geigy Incorporated, Los Angeles, CA) waveguide onto the 50nm thick gold SPR surface, with a limited range of incident angles. The light is reflected off the SPR surface and is eventually projected along a linear silicon photodiode detector array. Each of 256 pixels in the array corresponds to a narrow range of angles of incidence. The signal intensity from the array is used to quantify the resonance condition and thereby indicate the sample's index of refraction.

2.1.1 Spreeta™ electronics

For the work described here, a digital signal processor (TMX230F206, Texas Instruments, Dallas, TX) based electronics system was used. Each pixel of the array was read out individually using a 12-bit analog-to-digital (A/D) converter. These data were averaged on the DSP and this average was sent to a personal computer, where SPR curve analysis was performed. Data rates were limited to approximately one data point every 3 seconds to reduce sensor heating. Each data point consisted of an average of 16 individual measurements.

The short-term noise level for this system is approximately 1×10^{-6} refractive index units (1 standard deviation) and this generally defines the system sensitivity.

2.1.2 Flow cell

A flow cell was used in all of these experiments to enable relatively small fluid volumes to be brought to and from the active portion of the SPR sensing surface. Briefly, the body of the flow cell was a 1/4-inch thick Teflon™ block with holes drilled to accept 1/16-inch OD Teflon™ tubing (Upchurch Scientific, Oak Harbor, WA). A gasket (0.030-inch thick silicone), with a laser-cut channel (2mm x10 mm) was placed between the Teflon™ block and sensor surface. The channel was positioned so that it overlapped the active SPR region. Allowing for gasket compression, the flow cell volume for these studies was estimated to be approximately 10μL.

In general, liquids were applied to the sensor surface using a very simple peristaltic pump (Fisherbrand Variable Flow Peristaltic Pump) operating around 300μL/min.

2.1.3 Temperature compensation

The flow cell used in these studies was outfitted with a small glass bead thermistor (G43A78, Victory Engineering Corporation, Newark, NJ) which was placed directly in the flow cell channel. The response time of the thermistor was very fast (140ms), which allowed for the compensation of small changes in liquid temperature during refractive index measurements.

2.2 Disposable gold slides

These studies used disposable gold-coated glass slides as the SPR-active surfaces. These slides were prepared by depositing 2nm of chromium and 50nm of gold onto 0.008-inch thick borosilicate glass (Erie Scientific, Erie, PA) cut to fit over the

sensor surface. Both metal films were deposited by thermal evaporation (Varian model 3118, base pressure less than 2×10^{-7} Torr). The gold deposition rate was 0.8-1.0nm/sec, and the chromium deposition rate was 0.1-0.2nm/sec.

2.2.1 Gold slide pretreatment

In all of the experiments reported here, the gold-coated slides were stored in Fluoroware boxes in an uncontrolled laboratory ambient. Prior to the attachment of bio-films, the gold SPR surfaces were routinely cleaned to enhance assay reproducibility.

Since most cleaning procedures are temporary, we have elected to clean the gold surfaces immediately prior to use. In this work, the slides were given a treatment with an aqueous solution containing 0.12 N sodium hydroxide (NaOH) and 0.1% Triton X-100 (Sigma, St. Louis, MO). In our experience, this treatment leaves the gold surfaces hydrophilic and subsequent film growth is generally seen to be quite reproducible. Illustrations of this technique have been shown previously.¹¹

2.3 Conversion of Spreeta™ to a TNT sensor

Bovine Serum Albumin or BSA (Fluka, Buchs, Switzerland) was modified with trinitrobenzene sulfonic acid (Pierce, Rockford, IL) to introduce trinitrobenzene (TNB) groups. Briefly, 10mL of BSA (20mg/mL) was allowed to react at room temperature with 0.4mL of a 5% aqueous solution of trinitrobenzene sulfonic acid. After two hours the reaction mixture was dialyzed overnight against 2L of phosphate buffered saline (PBS pH 7.4). TNB-BSA coated slides were prepared when 0.5μL of TNB-BSA (20μg/mL in PBS) was spread across the surface of a cleaned gold-coated glass slide using a pipette tip. The slide was allowed to air dry, then placed into a 70°C vacuum oven for 15 minutes. The TNB-BSA covered slide was rinsed in HPLC-grade water and allowed to air dry before assembly into the Spreeta™ sensor. Optical coupling of the glass slide to the Spreeta sensor was accomplished by applying approximately 0.2μL of index matching liquid (#5040, Cargille Incorporated, Cedar Groves, NJ) onto the sensor surface and then placing the glass slide on top. The flow cell was assembled and mounted above the TNB-BSA slide. The flow cell held the glass slide firmly in place.

2.4 Soil sample preparation

A set of 180 spiked soil samples was prepared using three different soils: Fort Edwards clay, Fort Leonard Wood clay, and Ottawa sand. The clay soils were sieved through a 40-mesh sieve to remove larger aggregates. The sand was used as received. A 75.5 ± 0.5 mg portion of soil was carefully weighed into plastic micro-centrifuge tubes. Samples were spiked as shown in Table 1 to give concentrations of either military-grade TNT (TNT-MG), standard analytical reference grade TNT (TNT-SARM), 2,4-dinitrotoluene (2,4-DNT), 2,4-dinitrophenol (2,4-DNP), 2,4-dichlorophenoxyacetic acid (2,4-D), or ammonium nitrate (NH_4NO_3) of either 1ppm or 10ppm as indicated below.

The spiking solutions were prepared in HPLC-grade acetonitrile, except for the ammonium nitrate solution, which was prepared in reagent-grade water. The source of the military-grade TNT used in this test was a PMA-1A antipersonnel landmine (Yugoslavia). The precision of the spiking process was evaluated by spiking ten replicate 75μL aliquots into 1.00mL aliquots of acetonitrile and determining the spiked concentration by reversed-phase HPLC.¹² The relative standard deviation for 2,4-DNT was 4.5%. After spiking, the soil samples were placed in a fume hood to allow the solvent to evaporate. Samples were then cooled and shipped on blue ice by overnight carrier to Texas Instruments for analysis.

2.5 Extraction protocol

Upon arrival at Texas Instruments, the soil samples were stored overnight at 4°C and analyzed the following day. Soil extracts were prepared using a simple protocol. 0.75mg of soil was suspended in 1.15mL of PBS and 0.1% Triton X-100 in a 1.7mL micro-centrifuge tube. The soil was gently shaken in the solution for 5 minutes. The mixture was then centrifuged at 14,000 rpm for 5 minutes to pellet the soil particles. 1mL of the supernatant was removed by pipette and analyzed for TNT content.

Table 1. Sample spiking procedure for blind soil test

Analyte	Desired Concentration (mg/kg)	Spiking Solution Concentration (mg/L)	Spiking Solution Volume (μ L)
TNT-MG	1.0	1.10	75
TNT-MG	10.0	11.0	75
TNT-SARM	1.0	1.01	75
TNT-SARM	10.0	10.1	75
2,4-DNT	1.0	1.03	75
2,4-DNT	10.0	10.3	75
2,4-DNP	1.0	1.01	75
2,4-DNP	10.0	10.1	75
NH ₄ NO ₃	10.0	111	7.5
2,4-D	10.0	10.1	75
Blank spike	0.0	0.0	75
Blank (no spike)	0.0	-	0

2.6 Competition assay

In a direct binding SPR assay, an analyte is typically bound to an immobilized ligand on the SPR surface. The changes in local refractive index that occur during analyte binding describe the kinetics of interaction between the two molecules.

SPR detects changes in the surface mass concentrations of an analyte and therefore is generally more sensitive to large molecules. Since TNT is a rather small molecule (MW = 227 amu), direct binding assays of low concentrations of TNT to the SPR surface are not feasible. For this reason, a competition assay was developed.

2.6.1 TNT competition assay

In the competition assay format a dilute solution of a monoclonal antibody to TNT is passed across the sensor surface. The TNT antibody employed in this work was purchased from a proprietary vendor. The antibody is used as a large mass molecule (approximately 150 kDa) whose binding is competitively prevented by free TNT in solution. When the antibody binds to the TNB-BSA groups on the gold surface, a linear increase in refractive index is observed. Antibody solutions containing TNT show a reduced rate of antibody adsorption as well as a reduction in the amount of antibody bound. Although the TNT competition assay requires an antibody as reagent, the TNB-BSA surface itself is very robust and easily regenerated between measurements. A more detailed description of this is given in the Results Section.

3. RESULTS

Figure 1 shows a detailed view of the competition assay format for the detection of TNT. A steady baseline was first obtained in the running buffer, PBS and 0.1% Triton X-100. The sensor was then “calibrated” by injecting 1mL of free antibody, that is running buffer containing 7.6 μ g of anti-TNT antibody. Binding of anti-TNT antibody to the sensor surface is seen as a subsequent refractive index increase. The total refractive index increase following this 1mL injection ([TNT]=0) describes the maximum amount of anti-TNT antibody expected to bind. A reference was run periodically throughout these studies to verify that the regeneration process was not affecting the ability of the TNB-BSA surface to bind anti-TNT antibody. The “reference” peak height varied from slide to slide, but was generally within the range of 0.5×10^{-3} to 1.0×10^{-3} .

After the reference run in Figure 1, the antibody was then stripped from the sensor using a regeneration buffer, 0.12 N NaOH and 0.1% Triton X-100. A baseline was re-established in running buffer, and then antibody binding was examined in the presence of extracts from soil samples that contained either 0 or 1ppm TNT. The presence of TNT in the extract from the soil reduced the amount of antibody binding to the sensor surface.

Several features are worth noting in Figure 1. First, there is a reproducible spike that occurs when switching from running buffer to regeneration buffer. Interestingly, this effect is more pronounced when the refractive index of the two buffers is closely matched. The NaOH concentration in the regeneration buffer (0.12 N) was chosen to match the refractive index of PBS in the running buffer. The cause of this spike is as yet unidentified. Secondly, extracts from both Fort Edwards and Fort Leonard Wood clay samples cause an initial bulk decrease in the refractive index. This is most obvious in the third sample shown in Figure 3, where the antibody binding is totally inhibited by the TNT in the extract from sample #73. This seems to be due to the absorption of some of the Triton detergent by the Fort Edwards or Fort Leonard Wood clays. In extractions of Fort Leonard Wood soil with PBS alone, this bulk effect is eliminated. Thirdly, some non-specific binding occurs during injection of the second sample. Note that the antibody binding in the presence of the extract obtained from sample #72 ([TNT]=0) is slightly higher than that for antibody binding in running buffer.

Figure 1. Expanded view of the TNT detection data, samples #71, (free AB) #72 (FTED56) and #73 (FTED22) are shown. The reference contains anti-TNT in buffer and gives the zero TNT peak height for comparison to unknown samples. The negative sample shows antibody binding as well as some changes in the bulk refractive index, a drop when sample is introduced and a sharp rise when switching back to buffer to obtain the peak height. The positive sample shows the inhibition of anti-TNT binding due to the presence of free TNT in the sample. As in the negative sample, the drop in refractive index is due to a bulk change caused by the extraction of the clay.

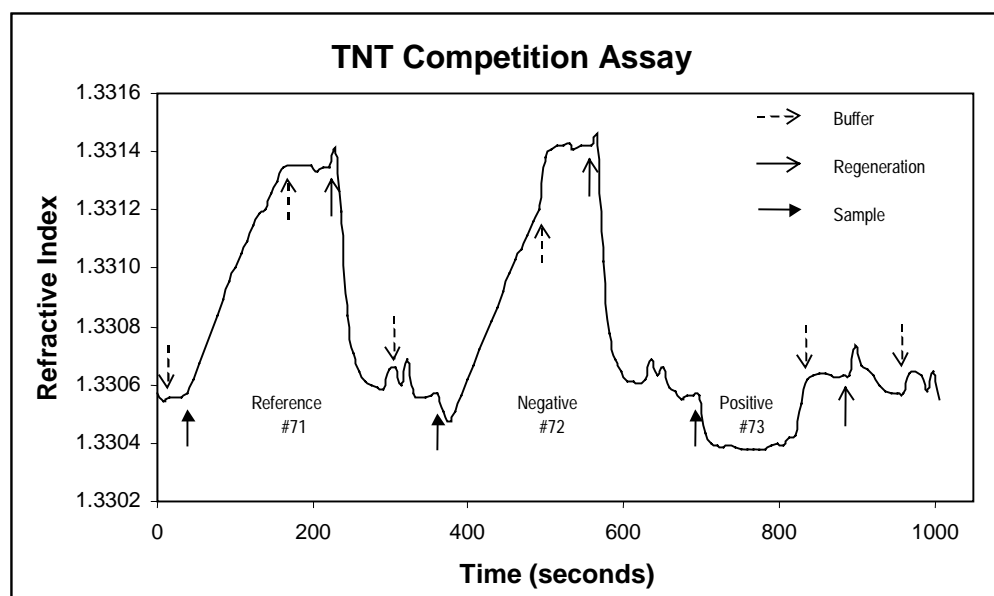


Figure 2. Samples 83-98 (Ab, Ottawa 2, Ottawa 6, Ottawa 7, Ab, Ottawa 56, Ottawa 13, Ottawa 14, Ab, Ottawa 55, Ottawa 57, Ottawa 15, Ab, Ottawa 16, Ottawa 17, Ottawa 20, where Ab is the reference). In contrast to the FTED or FTLW clay samples, there is no noticeable bulk refractive index change caused by the Ottawa sand. Tall peaks indicate no TNT, short peaks are positive for TNT and the intermediate height peak is due to interference from 1ppm TNT.

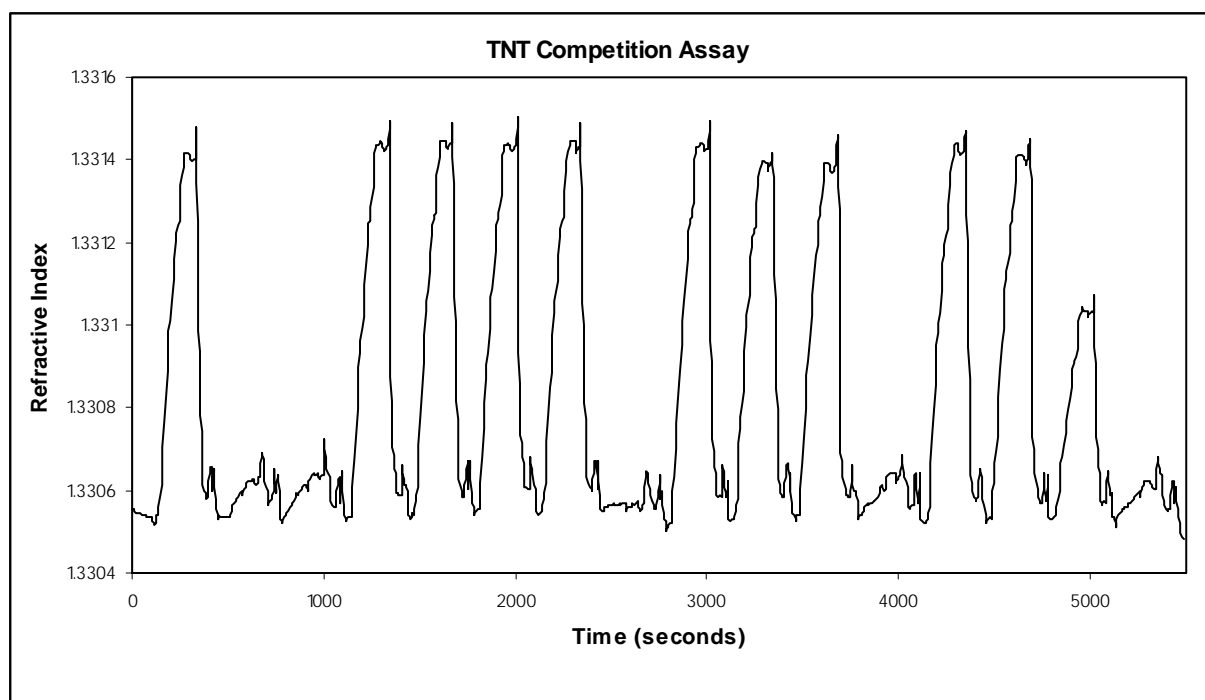


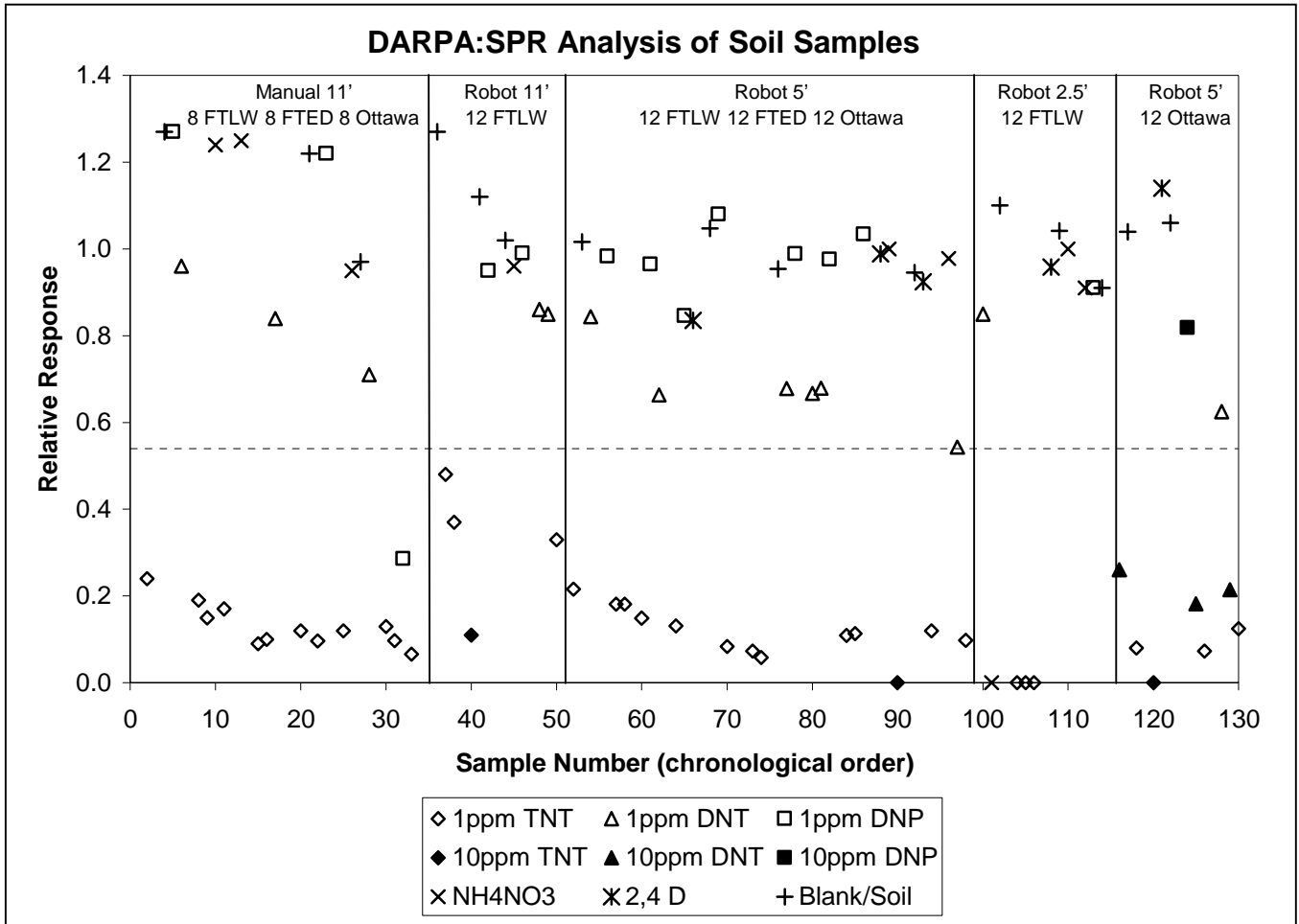
Figure 2 shows the competition assay repeated for 12 specimens of Ottawa sand, samples 83-98. This illustrates the reusability and reproducibility of the sensor response for a particular soil type. It should be noted that extraction of the sand specimens with PBS and 0.1% Triton does not result in the bulk effects noted for the Fort Edwards and Fort Leonard Wood clay samples.

The results of the entire double blind study for TNT detection are plotted in Figure 3 and the soil samples are listed in the order in which they were analyzed in Table 2. The graph in Figure 3 shows the relative response for each sample. The relative response is defined as the ratio of the refractive index increase observed upon sample binding to that observed upon free anti-TNT antibody binding. The reference samples for anti-TNT binding in running buffer are interspersed throughout the data and appear as missing data points. The relative response for a given sample is always calculated using the reference immediately preceding the data. The references are numbered and add to the sample total (130).

The first 34 samples were run manually. The total time to analyze one sample was 11 minutes, which included 3 minutes to obtain a baseline in running buffer, 2 minutes to inject the extract and antibody, 3 minutes to obtain another running buffer baseline, and 3 minutes for regeneration of the sensor.

The next sets of samples were analyzed with a robotic fluid dispensing system, the Automove 101 from Asymtek (Carlsbad, CA). The same 11 minute protocol was employed for samples 35-50. The assay time was then reduced to 5 minutes for samples 51-98 and samples 115-130 by adjusting the procedure. The new procedure included 1 minute to obtain a baseline in running buffer, 2 minutes to inject the extract and antibody, 1 minute to obtain another baseline in running buffer, and 1 minute for regeneration of the sensor. Samples 99-114 were run with a 2.5 minute assay. The 2.5 minute assay included 30 seconds for obtaining baselines in the running buffer, 1 minute for injection of the extract and antibody, and 30 seconds for regeneration of the sensor surface.

Figure 3. Results of the double blind study for TNT detection.



4. DISCUSSION

4.1 Sample composition

It is instructive to compare the samples studied. The samples were of three soil types, Fort Leonard Wood clay, Fort Edwards clay or Ottawa sand. Multiple soils were analyzed because it is known that a number of physical and chemical properties of soil affect the sorption and therefore the ease of extraction of explosive chemicals. Some of these properties are the soil density, porosity, moisture content and particle size.⁸ Explosive compounds cannot be extracted from some soils, using common laboratory methods.¹³

All three soil types were spiked with either, TNT (1ppm or 10ppm), DNT (1ppm or 10ppm), NH_4NO_3 (10ppm), 2,4 D (10ppm), 2,4 DNP (10ppm) or no analyte (blank). Compounds other than TNT were included for various reasons. DNT and 2,4 DNP for example, are chemically similar to TNT. DNT is a breakdown product of TNT and a major impurity in production grade TNT.¹⁴ DNT is not expected to be present in soils naturally and therefore is not considered an interferant for the purposes of this work. 2,4-DNP however might be a potential interferant, binding to the monoclonal antibody used in the competition assay and resulting in the false detection of TNT. Other potential interferants include fertilizers and pesticides. Ammonium nitrate is one widely used and inexpensive fertilizer. 2,4 D is a common herbicide.

4.2 Sample scoring

It was decided that points above a horizontal dotted cutoff line would be scored negative for TNT, and points below scored positive for TNT. Statistical analysis of the data in Figure 3 gave an average positive response ratio of 0.14 ± 0.1 for the 1ppm TNT data, and an average negative response ratio of 0.90 ± 0.27 (all data except 1 & 10ppm TNT). A mean cutoff of 0.52 would have biased toward false positives (1.4 versus 3.8 sigma). Given the sample space tested, the chance of a false positive would have been 8% and the chance of a false negative, 1 in 14,000 trials.

Most of the assay interference we observed appeared to be the result of cross reactivity of the anti-TNT antibody with DNT. Removing the DNT data gave an average negative response ratio of 0.98 ± 0.22 and an average cutoff of 0.56. This moved the mean values for positive and negative response ratios to 1.9 and 4.2 sigma from the cutoff. A mean cutoff of 0.56 would still bias toward false positives, 3%, but the false negative rate would be reduced to 1 in 75,000 trials. Choosing the average of these two cutoffs at 0.54 resulted in no false positive responses for 1ppm DNT. Therefore any sample with a relative response greater than 0.54 was scored negative for TNT and any sample with a relative response less than 0.54 was scored positive for TNT.

4.3 Assay performance

As shown in Figure 3, we achieved a 100% detection probability for soils containing 1 or 10ppm TNT with minimal false positives. There was very little interference from NH_4NO_3 , 2,4D or DNP at the 10ppm level. The antibody used here did cross react with DNT, such that a 10ppm DNT concentration gave a signal slightly less than 1ppm TNT. All the 1ppm DNT samples were above the 54% cutoff line.

One solution to the DNT interference would be to select a monoclonal antibody that distinguished between TNT and DNT. Another approach is to implement a second channel in the SPR sensor that has an anti-DNT antibody. In this way, TNT and DNT could be distinguished.

Two of the samples that contained 1ppm TNT (#37 and #38) came very close to the 54% cutoff line. These data points were obtained on the robot with a new TNB-BSA slide in the sensor. From the data in Figure 3 there appears to be a conditioning of the new TNB-BSA slide as the positive samples slowly moved away from the cutoff line at 0.54 and testing progressed. A non-specific binding component in the soil may have been the cause of the high relative response. It is important to note that the blank samples run at the same time exhibit the same behavior. This seasoning issue is obviously an important one to understand.

The glaring false positives were (#32 and #101), a DNP and ammonium nitrate sample, respectively. Since the many other DNP and ammonium nitrate samples did not give false positive signals, we tend to believe there was cause other than assay interference.

4.4 Assay Relevance

It is important to note that the data presented herein was gathered using laboratory-spiked samples, and not actual field samples. There are differences in the way in which TNT adsorbs to field and spiked soils. There are also differences in the ease in which TNT can be extracted from field or spiked soils. The applicability of our TNT extraction procedure to field samples must be addressed. Furthermore, recent studies of actual TNT concentrations in the soils around mines generally place the level of TNT well below 1ppm. In the field, much of the TNT decomposes through photo-degradation or microbial processes. The actual value of TNT present depends on many parameters including the condition and age of the mine, the mine casing material and weathering. Therefore, to make this SPR-based competitive assay more relevant to actual land mine detection, it is instructive to consider what steps can be taken to increase the sensitivity.

4.4.1 Extraction efficiency

To determine the actual efficiency of the soil extraction process, we quantified extractions performed in water, methanol or acetone by reversed phase high performance liquid chromatography (RP-HPLC). All samples contained 150mg of Fort Leonard Wood Clay spiked with TNT at 10 μ g/mL. The water and methanol extracts were prepared in an identical matrix of 50:50 water:methanol. The acetone extracts were prepared in a 3:1 water:acetone matrix. The extracts were analyzed against standards prepared in an identical matrix. We found that methanol and acetone were nearly 100% effective in extracting TNT from the soils. The aqueous extracts contained only 40% of the available TNT. The mean concentrations of TNT for the extracts were computed on a mg/kg soil basis. They were 4.09 (\pm 0.59) for the water extractions, 8.09 (\pm 0.34) for the acetone extractions and 9.24 (\pm 0.62) for the methanol extractions. The superior extraction capability of methanol for TNT agrees with extraction results presented elsewhere.¹⁵ While aqueous extracts are not as efficient at extracting TNT, they are generally best suited for immunoassays.

4.4.2 Sensitivity

To evaluate the overall sensitivity, it is important to consider the stoichiometry of the assay. An anti-TNT antibody concentration of 7.8ug/ml is equivalent to 52nM. At 1ppm, assuming all of the TNT in a 75mg soil sample is successfully extracted into the aqueous buffer, the TNT concentration would be 330nM. The TNT antibody being bivalent, binds two molecules of TNT per antibody, so essentially there is a 3-fold molar excess of available TNT versus antibody. However the efficiency of the soil extraction in water, described above, suggests that there is actually only a 1.25-fold molar excess of TNT compared to antibody.

Given the experimental conditions employed here, the competition assay would have been performing close to its detection limit. Subsequent experiments on spiked soil samples agreed with this conclusion indicating that the lower limit of detection for these assay parameters was approximately 300ppb.

There are several steps that could be taken to increase the sensitivity of this competition assay. The amount of soil sampled could be increased relative to the volume of solvent used, effectively increasing the TNT concentration of the liquid sample. Reducing the antibody concentration would also bolster sensitivity by reducing the number of TNT molecules needed to provide a given percentage drop in antibody binding, as compared to a reference run. It is also likely that a boost in performance could be obtained by the use of antibodies directed specifically to TNT breakdown products such as 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene.

5. CONCLUSIONS

This study illustrated that a SPR sensor could be used effectively to screen soil samples for TNT and related materials using a simple bioassay and aqueous soil extracts. The estimated limits of detection of 1ppm TNT (1mgTNT/kg soil) were in agreement with the stoichiometry of the assay and the amount of TNT extracted from the soil. Methods to increase the sensitivity of the assay were discussed.

Sample preparation for the TNT assay was minimal and fouling of the bio-film-sensing surface was generally not a problem. However, seasoning of the sensor surface before sample analysis is advised.

Interferants were not a problem for this TNT competition assay except in the case of 10ppm DNT. Since DNT is a decomposition product of TNT and not expected to be naturally present in soil, we do not perceive this as a true disadvantage.

Clearly, for such a methodology to be useful in the field, a soil collector/extractor is required. The collector/extractor would have to perform its duty rapidly and efficiently. Given such a device and a suitable liquid sample, it is our belief that the Spreeta™ sensor employed here is a viable method for detecting TNT and other explosives from soils.

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7. REFERENCES

1. G. Strada, "The horror of land mines," *Sci. Am.* **274**, pp. 40-45, 1996.
2. A. M. Rouhi, "Land mines: horrors begging for solutions," *Chemical and Engineering News* **75**, pp. 14-22, 1997.
3. S. Y. Rabbany, B. L. Donner, F. S. Ligler, "Optical immunosensors," *Crit. Rev. Biomed. Eng.* **22**, pp. 307-346, 1994.
4. P. Schuck, "Use of surface plasmon resonance to probe the equilibrium and dynamic aspects of interactions between biological macromolecules," *Annu. Rev. Biophys. Biomol. Struct.* **26**, pp. 541-566, 1997.
5. D. J. O'Shannessy, "Determination of kinetic rate and equilibrium binding constants for macromolecular interactions: a critique of the surface plasmon resonance literature," *Curr. Opin. Biotechnol.* **5**, pp. 65-71, 1994.
6. U. Jönsson, L. Fägerstam, B. Ivarsson, K. Lundh, S. Löfås, B. Persson, H. Roos, I. Rönnerberg, S. Sjölander, E. Stenber, R. Ståhlberg, C. Urbaniczky, H. Östlin, M. Malmqvist, "Real-time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology," *BioTechniques* **11**, pp. 620-627, 1991.
7. A. Szabo, L. Stolz and R. Granzow, "Surface plasmon resonance and its use in biomolecular interaction analysis (BIA)," *Curr. Opin. Struc. Biol.* **5**, pp. 699-705, 1995.
8. J. M. Phelan, S. W. Webb, "Environmental fate and transport of chemical signatures from buried land mines – screening model formulation and initial simulations," Report SAND97-1426, Sandia National Laboratories, Albuquerque, NM, 1997.
9. J. Melendez, R. Carr, D. U. Bartholomew, K. Kukanskis, J. Elkind, S. Yee, C. Furlong, R. Woodbury, "A commercial solution for surface plasmon sensing," *Sensors and Actuators B* **35**, pp. 1-5, 1996.
10. R. G. Woodbury, C. Wendin, J. Clendenning, J. Melendez, J. Elkind, D. Bartholomew, S. Brown, C. E. Furlong, "Construction of biosensors using a gold-binding polypeptide and a miniature integrated surface plasmon resonance sensor," *Biosens. Bioelectron.* **13**, pp. 1117-1126, 1998.
11. J. L. Elkind, D. I. Stimpson, A. A. Strong, D. U. Bartholomew, J. L. Melendez, "Integrated analytical sensors: the use of the TISPR-1 as a biosensor," *Sensors and Actuators B*, submitted.
12. U.S. Environmental Protection Agency, "Nitroaromatics and nitramines by high performance liquid chromatography," SW-846 Method 8330.
13. M. Fisher, C. Cumming, M. la Grone, R. Taylor, "An electrostatic particle sampler and chemical sensor system for land mine detection by chemical signature," in *Detection and Remediation Technologies for Mines and Minelike Targets III*, Abinash C. Dubey, James F. Harvey, J. Thomas Broach, Editors, Proceedings of SPIE **3392**, pp.565-574, 1998.
14. T. F. Jenkins, M. E. Walsh, "Development of field screening methods for TNT, 2,4-DNT and RDX in soil," *Talanta* **39**, pp. 419-428, 1992.
15. T. F. Jenkins, C. L. Grant, "Comparison of extraction techniques for munitions in soil," *Anal. Chem.* **59**, pp. 1326-1331, 1987.