Journal of Biosensors & Bioelectronics
ISSN: 2155-6210

Wang et al., J Biosens Bioelectron 2012, 3:1
DOI: 10.4172/2155-6210.1000115

Research Article Open Access

Sensitive Detection of TNT using Competition Assay on Quartz Crystal Microbalance

Jinghui Wang, Mohammed AlMakhaita, Sibani Lisa Biswal* and Laura Segatori*

Department of Chemical and Biomolecular Engineering, Rice University, Houston, TX 77005, USA

Abstract

Currently available TNT sensors are characterized by high sensitivity, but low specificity, which limits the detection of TNT in dirty environments. We report here a TNT sensor designed to measure the displacement of a TNT-specific antibody by quartz crystal microbalance (QCM). This sensor combines high sensitivity of detection (0.1 ng/mL) with the ability to distinguish TNT from molecules with similar chemical properties. Particularly, the reliability of this method for the detection of TNT in dirty environments was investigated by using fertilizer solution and artificial seawater. Instead of measuring actual binding of TNT, the method described is based on the displacement of an anti-TNT antibody, which allows quantifying the concentration of TNT in solution with higher sensitivity. In addition, by utilizing the rate of antibody displacement, the detection time is significantly decreased from hours, which would be necessary to measure the frequency change at equilibrium, to minutes. A Langmuir kinetic model was used to describe the molecular interactions on the surface of the sensor and to establish a standard curve to estimate on-site TNT detection. In summary, QCM detection of anti-TNT antibody displacement provides a method for rapid detection of TNT with high sensitivity and specificity.

Keywords: TNT detection; Competition assay; Quartz crystal microbalance; Langmuir kinetic model

Introduction

The rapid and reliable detection of explosives has gained increasing attention, due to health and public safety reasons [1]. Most types of currently used explosives are toxic to living systems, even when present in trace amounts. This study focuses on the detection of 2,4,6-trinitrotoluene (TNT).

A variety of technologies have been developed for the detection of TNT. They can be broadly classified as physical, chemical and biological methods, based on the detection mechanism and output signal. Physical methods, such as laser, mass spectroscopy and NMR [2-4], allow achieving high sensitivity of detection, but involve time consuming and costly procedures. Particularly, TNT concentrations as low as 1pg/mL can be detected in vapor phase with low false positive signals using laser photoacoustic spectroscopy [2]. The specificity of chemical methods, including electrochemical sensors [5,6] and fluorescence spectrophotometry [7-9], is relatively low. Fluorescence spectrophotometry is based on the electron deficiency of TNT, which as many nitroaromatic compounds, functions as electron acceptor and causes quenching of a number of photoluminescent [10], fluorescent [11], and phosphorescent [12] materials by electron transfer. Among these materials, fluorescent polymers were first reported to allow detecting saturated TNT vapor (0.1ng/mL) within seconds [13]. The performance of fluorescence spectrophotometry was later improved using mesostructured silica films [14], nanocrystals [15], and quantum dots [16]. Particularly, the limit of detection for TNT was reduced to ~0.023ng/mL using a hybrid material composed of gold nanorod and quantum dots. This method, however, exhibits relatively low specificity, which prevents distinguishing TNT from other nitroaromatic compounds with similar chemical properties [17]. Biological methods typically present enhanced specificity due to the use of TNT specific molecules, such as antibodies [18,19], and molecularly imprinted polymers (MIPs) [20,21]. Most of these reported sensors lack data comparing their performance in a dirty environment. The purpose of this study is to develop a rapid and accurate sensor that combines the high sensitivity of chemical methods with the high specificity of biological methods for the detection of TNT in aqueous solutions containing similar molecules.

One of the critical issues in the development of a TNT sensor is the small size of the TNT molecule that often precludes high sensitivity of detection at low concentrations. To overcome this limitation we designed a sensor based on the detection of a TNT-specific antibody. Particularly, the concentration of TNT in solution was measured by quantifying the antibody displacement from the surface by TNT, a strategy that allows amplifying the output signal compared to the signal associated with direct detection of TNT [22-25]. The detection of TNT using displacement assay was previously reported using Enzyme-Linked Immunosorbent Assay (ELISA) [26-28], which is used as comparison in this study. In addition, the method of antibody displacement was also previously used for TNT detection in a flow system (microcapillary) [29].

A number of techniques have been used for the detection of TNT including surface plasmon resonance (SPR) [30] and fluorescence resonance energy transfer (FRET) [31]. In this study we used quartz crystal microbalance (QCM) couple with the antibody displacement method described above. QCM has been widely used as biosensor, in studies of affinity estimation [32] and polymer conformational changes [33], due to its high sensitivity, label-free, real-time measurements,

***Corresponding authors:** Sibani Lisa Biswal, Department of Chemical and Biomolecular Engineering, Rice University, Houston, TX 77005, USA, Tel: +1 (713) 348-6055; Fax: +1 (713) 348-5478; E-mail: biswal@rice.edu

Laura Segatori, Department of Chemical and Biomolecular Engineering, Rice University, Houston, TX 77005, USA, Tel: +1 (713) 348-3536; Fax: +1 (713) 348-5478; E-mail: segatori@rice.edu

Received September 09, 2011; **Accepted** December 1, 2011; **Published** December 25, 2011

Citation: Wang J, AlMakhaita M, Biswal SL, Segatori L (2012) Sensitive Detection of TNT using Competition Assay on Quartz Crystal Microbalance. J Biosens Bioelectron 3:115. doi:10.4172/2155-6210.1000115

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portability, and ease of operation [34]. In this study, we describe the development of a QCM based sensor for TNT detection that allows measuring TNT with high sensitivity in pure solution as well as in dirty environments, such as fertilizers and seawater [35]. The kinetics of antibody displacement during the detection process was studied through a Langmuir adsorption based model.

Materials and Methods

Materials

2,4,6-Trinitrotoluene (TNT), 1,3,5-Trinitrobenzene (TNB), 2,6-Dinitrotoluene (DNT), 2-Amino-4, 6-Dinitrotoluene (2-a-DNT), and ovalbumin were purchased from *Sigma-Aldrich*. Anti-TNT monoclonal antibody A1.1.1 was from *Strategic Diagnostics*. HRP conjugated anti-mouse antibody was purchased from *Assay Designs*. 2,4,6-trinitrobenzene sulfonic acid (TNBS) and Dithiobis[succinimidyl propionate] (DSP) were from *Pierce*. TMB substrate was purchased from *BioLegend*.

Synthesis of TNB-ovalbumin complex

The TNB-ovalbumin complex was prepared by conjugating the sulfonic group of 2, 4, 6-trinitrobenzene sulfonic acid (TNBS) to the primary amines of ovalbumin molecules as previously described [36]. Briefly, a solution of 10.2 mM TNBS and 0.67mM ovalbumin in PBS (pH 8.0) was stirred at 30rpm for one hour at room temperature. The reaction product was dialyzed overnight against PBS to eliminate free TNBS and stored at -80 °C until use.

Preparation of TNT and TNT analogs

Acetonitrile was evaporated from the stock solution of TNT (1000 µg/mL) and TNT analogs. Then PBS buffer, fertilizer solution or seawater, was used to dissolve them before use. Commercially available fertilizer powder was dissolved in PBS buffer at a concentration of 1mg/mL (0.1% w/v), and the pH adjusted to 7.4. Artificial seawater was prepared by dissolving 100% natural sea salt in deionized water (26.7g/L) to obtain a solution containing the same concentration of sodium as natural seawater (0.469 mol/kg).

ELISA

100 µL of TNB-ovalbumin complex at 10 µg/mL in 0.1M sodium bicarbonate were added to each well of a 96-well plate and plates incubated overnight at 4°C. After washing with 0.1% PBST, PBS 4% milk (200 µL/well) was added to block uncoated sites. TNT or TNT analogs at concentration specified in each experiment and mouse anti-TNT antibody (0.5µg/mL) were added to each well and plates incubated for ~2 hours with gentle shaking. After washing with 0.1% PBST, HRP-conjugated goat anti-mouse antibody (100 ng/mL) was added to each well, plates incubated for 1 hour, and washed again. 100µL/well of TMB substrate were added, and, after 10 minutes, 50 µL/well of 1M phosphoric acid were added to stop the reaction. The absorbance at 450 nm was measured with a *GeneMate UniRead 800* plate reader. The cross reactivity (CR) [37] of the anti-TNT antibody with each compound was evaluated as follows:

$$CR = \frac{C_0}{C} \times 100\% \quad (1)$$

where C_0 is the concentration of TNT upon 50% of antibody displacement, and C is the concentration of compound used to achieve 50% displacement.

Functionalization of the crystal surface

Crystals were washed with a mixture of hydrogen peroxide and ammonia hydroxide at 75 °C, and UV-ozone cleaner (*novascan)* under 5 psi oxygen. Dithiobis [succinimidyl propionate] (DSP) was used as cross-linker to immobilize TNB-ovalbumin complex on the gold surface of crystals. Crystals were first immersed in DSP (1 mg/mL in DMSO) for 30 min, and then in TNB-ovalbumin complex (100 µg/mL) for 2 hour to form a "sandwich" structure: "Au↔DSP↔TNB-ovalbumin", on the surface. Crystals were incubated in 1M Tris overnight to block uncoated sites.

QCM assay

The QCM system used in this study is a Q-sense E4 system (Q-Sense, Västra Frölunda, Sweden), which measures changes in mass and related viscoelastic properties. The AT-cut QCM crystal used has a resonance frequency of 5 MHz. Using Sauerbrey equation [38], 1Hz frequency change can be converted to a mass change of 17.7 ng/cm^2 on the crystal surface. The viscoelastic properties can be obtained from energy dissipation measured by the decay of oscillation [39]. After immobilization of TNB-ovalbumin complex, anti-TNT antibody and solutions of TNT or TNT analogs were flowed sequentially at 50 µl/min at 25°C.

The frequency change measured after addition of TNT was divided by the frequency change caused by the addition of antibody (Δf_{dis}). Δf_{dis} obtained from each compound tested was divided by the Δf_{dis} of the control sample (crystal without TNT or TNT analog), and the new parameter obtained, the normalized frequency change (Δf_N), was used for the data analysis.

$$\Delta f_{dis} = \frac{\Delta f_{TNT}}{\Delta f_{Ab}} \ , \ \Delta f_N = \frac{\Delta f_{dis}\ of\ each\ compound}{\Delta f_{dis}\ of\ control\ sample} \quad (2)$$

The ratio of frequency change over a chosen time interval, defined "quick" slope *k*, was calculated as follows:

$$k = \frac{\Delta f_N}{\Delta t} \quad (3)$$

where Δf_N is the normalized frequency change during the time interval Δt.

Mathematical modeling of antibody displacement on QCM

The displacement of antibody on QCM can be simulated by assuming a Langmuir isotherm model. The deduction steps of the model have been previously reported [40,41]. After the displacement step reaches equilibrium, the following equation describes the relationship between TNT concentration and the frequency change measured.

$$\frac{[TNT]}{\Delta f} = \frac{[TNT]}{\Delta f_{max}} + \frac{1}{\Delta f_{max} K_A} \quad (4)$$

where Δf is the frequency change at a given concentration of TNT, Δf_{max} is the maximal frequency change when the antibody is completely displaced, and KA is the binding affinity constant (Equation S-4). Δf_{max} and KA were calculated from the plot of [TNT]/Δf with respect to [*TNT*] (Equation 4). In order to simulate the data obtained from QCM, the kinetics of antibody displacement was derived as described in the Supporting Information. Briefly, the expression describing the formation of the antibody-TNT complex (Equation S-5) [41] was integrated (Equation S-6), to calculate the forward reaction rate k_A. An ODE describing the change of Δf as a

function of time was obtained from the expression of k_A (Equation 5), and analyzed in Matlab. The model derived was used to analyze the experimental data, and predict the kinetics of displacement under different conditions, such as different pH and temperature.

$$\frac{d\Delta f}{dt} = k_A[TNT]\Delta f_{\max} - (k_A[TNT] + \frac{k_A}{K_A})\Delta f \quad (5)$$

Results and Discussion

Detection of TNT through antibody displacement by ELISA

The anti-TNT monoclonal antibody A1.1.1 was chosen because of its high binding specificity to TNT [42] compared to other nitro aromatic compounds with similar structures [37]. ELISA analyses were first conducted to evaluate the feasibility of the displacement assay and determine the affinity and limit of detection of this antibody with a currently well-established technique. The principle of the displacement assay is based on the ability of the anti-TNT antibody to cross react with TNT analogs. TNB, which was previously reported to bind to this anti-TNT antibody with low affinity [37] was used as reference. The ability of TNT and other TNT analogs (including TNB) to displace the antibody from TNB was evaluated. Measurements of antibody displacement are reported in Figure 1 using TNT, TNB, DNT, and 2-a-DNT (Table 1). TNT was observed to cause the maximum antibody displacement and the limit of detection was estimated to be 1ng/mL.

The anti-TNT antibody used was isolated based on its high specificity to TNT, but, similarly to most antibodies, it displays cross-reactivity to compounds structurally similar to TNT. To quantify the binding affinity, the cross reactivity (CR) of the antibody for each compound was calculated as shown in Equation 1 (Table 2). The results obtained illustrate the antibody relative affinity: TNT>TNB>2-a-DNT>DNT, and confirm previously reported measurements [27,37,43,44].

Development of a QCM based displacement assay for TNT detection

The method developed for QCM detection of TNT is based on the principle of antibody displacement described above. The TNB-ovalbumin complex was first immobilized onto the surface of the crystal. The surface was then saturated with anti-TNT antibody, and subsequently a flux of TNT or TNT analogs was used to displace the antibody. The change in frequency was recorded until a plateau was reached, indicating maximum displacement of antibody. Because the molecular weight of the antibody is much greater than that of the TNT molecule, detecting the frequency change caused by the antibody displacement, rather than that associated with the binding of TNT, gives rise to significant amplification of the detection signal. This enables the sensor to achieve higher sensitivity and lower limit of detection. Control studies were conducted to measure the frequency change caused by flowing TNT on a crystal coated with TNB-ovalbumin but without anti-TNT antibody, which resulted in signal indistinguishable from the background (e.g. the frequency change associated with flux of PBS buffer on to a TNB-ovalbumin coated crystal), indicating the absence of non-specific binding (data not shown).

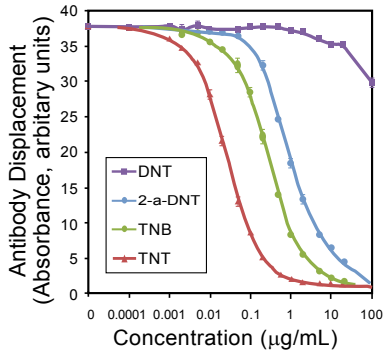


Figure 1: ELISA measurements of anti-TNT antibody displacement from TNB by TNT and TNT analogs. Compounds were tested at concentrations ranging from 0.1ng/mL to 100µg/mL and anti-TNT antibody A1.1.1 at 10µg/mL. Data points were obtained from at least three independent experiments and normalized to the signal measured from control samples containing only antibody. The detection limit for each molecule corresponds to the lowest concentration that causes loss of absorbance.

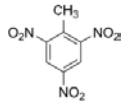
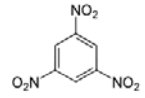
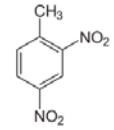
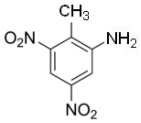
Name	Chemical Structure
2,4,6-Trinitrotoluene (TNT)	
1, 3, 5-Trinitrobenzene (TNB)	
2, 6-Dinitrotoluene (DNT)	
2-Amino-4, 6-Dinitrotoluene (2-a-DNT)	

Table 1: Chemical structure of TNT and TNT analogs used in this study.

Compound	Concentration upon 50% of antibody displacement, µg/mL	*CR, %*
TNT	0.025	100
TNB	0.3	8.3
2-a-DNT	0.94	2.6
DNT	>100	<0.02

Table 2: Cross reactivities for the compounds using antibody A1.1.1.

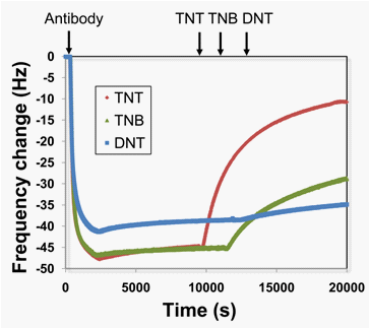


Figure 2: QCM measurements for the detection of TNT. Antibody displacement from TNB by TNT and TNT analogs. Anti-TNT antibody (10µg/mL) was immobilized on the crystal surface and three compounds (10µg/mL, TNT: blue line; TNB: red line; DNT: brown line) were tested. The arrows represent the time points when TNT and its analogs were added. Results are representative of three independent experiments.

The antibody displacements by TNT and two analogous compounds, TNB and DNT, at a concentration of 10 μg/mL were tested by QCM (Figure 2). A sharp decrease of frequency (around 45 *Hz* on average) is observed upon binding of the antibody to the TNB-ovalbumin complex on the crystal surface, which corresponds to a mass change of 506.25ng from the Sauerbrey equation. The number of antibody molecules attached on sensor surface is 2.03×10^{12}. Given the size of crystal (9 mm diameter) and ovalbumin molecule (6.1 nm diameter), we estimated that 2.18×10^{12} molecules of ovalbumin are immobilized on the sensor surface. Hence, we can assume that virtually all TNB-ovalbumin molecules form a complex with the antibody. After immobilization of the antibody, the solution of compound was flowed on to the crystal and the displacement of antibody was monitored (Figure 2). To minimize the variability of surface immobilization, (frequency change of TNT / frequency change of antibody) was used in the data analysis (Equation 2). The displacement caused by TNT was about 3-fold higher than that observed using DNT. The extent of displacement (the magnitude of frequency change) obtained at equilibrium reflects the binding affinity of each compound for the anti-TNT antibody. The antibody displacement measured by QCM is in agreement with the relative affinity of the three compounds calculated by ELISA: TNT>TNB>DNT.

The results presented above suggest that this sensor allows distinguishing TNT from molecules with similar chemical structure when they are present in solution at similar concentrations. However, the frequency change associated with a solution containing low TNT concentration is expected to be similar to that of a solution containing high TNB concentration. To address this issue we considered the energy dissipation. The change of energy dissipation D was related to the change of frequency F, thereby removing the time dependency of the data [45]. The slope in ΔD versus Δf plot indicates different states (conformations) of proteins [46] (anti-TNT antibody in this study). Results from QCM revealed that 1μg/mL TNT (Δf =26.65Hz) induces a frequency change similar to 10 μg/mL TNB (Δf=28.85Hz). However, the $\Delta D/\Delta f$ ratio of 1μg/mL TNT ($\Delta D/\Delta f = 0.0051\pm0.0011\times10^{-6}$/Hz) was significantly lower than that of TNB ($\Delta D/\Delta f = 0.0111 \pm 0.0007\times10^{-6}$/Hz), as easily appreciated comparing the slopes of the ΔD versus Δf plot reported in Figure 3, which indicates that TNT can be distinguished from TNB even when they induce similar signals on QCM. In sum, we demonstrated that QCM can be used to effectively distinguish TNT from other molecules with similar chemical structure.

Limit of detection of the QCM based TNT sensor

The QCM based sensor described here allows detecting TNT with high specificity in solution, and distinguishing it from molecules with similar chemical structure. We next investigated the detection limit (sensitivity) of this sensor by testing solutions of TNT ranging from 0.1ng/mL to 10μg/mL. The extent of antibody displacement was proportional to the concentration of TNT in solution. The data analysis was based on the use of the normalized frequency change Δf_N (Equation 2), which was calculated by dividing the Δf_{dis} of each compound by Δf_{dis} of a control sample without TNT. Thus the resulting values are always equal or greater than one. The normalized frequency changes of TNT at different concentrations are reported in Figure 4A. The lowest detectable concentration of TNT in this assay was 0.1ng/mL

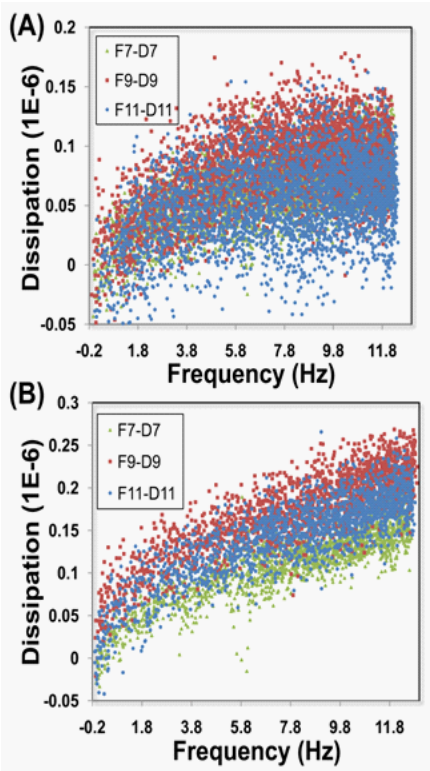


Figure 3: ΔD - Δf plot for TNT at 1μg/mL (A) and TNB at 10μg/mL (B). Colored points represent different overtones of frequency in QCM measurement. The slopes are obtained from the trend lines of these points. And the difference in slopes distinguishes TNT from its analogs.

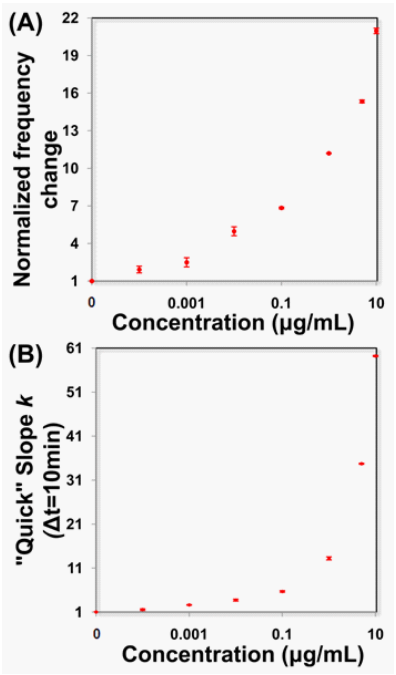


Figure 4: (A) Limit of detection of the QCM based TNT sensor. A solution of TNT at concentrations ranging from 0.1ng/mL to 10μg/mL was flowed on to the QCM crystal and the normalized frequency change was calculated. The data points reported were obtained at equilibrium from at least three independent experiments. (B) "Quick" slope analysis for rapid detection of TNT. The concentration of TNT in the solutions analyzed ranged from 0.1ng/mL to 10μg/mL, and the time interval chosen was 10 minutes. The data points reported were obtained from at least three independent experiments.

(p<0.01), which is one order of magnitude lower than what previously determined by ELISA (1ng/mL), demonstrating the superior properties of a QCM based sensor for applications in the field.

Accelerated detection using the rate of antibody displacement

The analysis described above is based on the measurements of the frequency change at equilibrium caused by the displacement of anti-TNT antibody after the addition of TNT, which usually requires several hours. This time scale is typically not considered practical for rapid on-site detection. Hence, we introduced a new parameter, the "quick" slope k (Equation 3), which allows estimating the dependence of the rate of displacement on the concentration of TNT.

The "quick" slope k was calculated for solutions of TNT ranging from 0.1ng/mL to 10μg/mL, and a time scale of 10 minutes (Figure 4B). The "quick" slope was observed to increase with the concentration of TNT even at low TNT concentrations (0.1 ng/mL, p<0.01). Therefore, the "quick" slope k can be used to reliably quantify the detection of TNT in solutions of different TNT concentrations (0.1ng/mL - 10μg/mL) while considerably decreasing the time of detection (~10 minutes).

Detection of TNT in dirty environments

The experiments described above were conducted using solutions of TNT in PBS buffer, which is an ideal solvent and hardly recapitulates the conditions of on-site analysis. Thus, in an attempt to evaluate the reliability and robustness of this sensor for use in the field, solutions of TNT crowded with molecules with similar chemical structure, which might interfere with the detection, were tested. Particularly, we used a solution of commercially available fertilizer, which contains nitrogenous compounds with chemical reactivity potentially similar to TNT and TNT analogs, and seawater [35], which represents a commonly contaminated environment.

The rate of displacement and normalized frequency change at equilibrium for solutions of TNT in PBS buffer, in fertilizer, and in artificial seawater were found to be comparable (data not shown), demonstrating the robustness of this detection method. We then investigated the limit of detection of TNT in dirty environments and compared it to PBS (0.1ng/mL) (Figure 5). The normalized frequency changes obtained from the fertilizer solution and seawater were 1.50±0.15 and 1.56±0.16, respectively (p<0.05), which are comparable to the normalized frequency change measured using PBS (1.92±0.27, p<0.01), indicating that the QCM based detection of TNT is not limited by the composition of the solution.

Mathematical modeling and calculation of the binding affinity constants

The displacement process can be modeled with a Langmuir isotherm model, which was previously used to describe antigen-antibody interactions on a surface [47,48]. The binding affinity constant K_A refers to the relative affinity of the anti-TNT antibody to a specific compound and was determined based on Equation 4 (Figure S-1). The maximum frequency change Δf_{max} caused by antibody displacement and TNT binding affinity constant K_A are 0.73 ± 0.05 and 5.09 ± 0.61, respectively. The positive reaction rate k_A (Equation S-5, Figure S-2) was (5.247 ± 0.027)×10^{-5}. The data obtained with solutions of TNB and DNT were analyzed in the same fashion and results are reported in Table 3. The values of both Δf_{max} and K_A represent the relative affinity of each molecule for the anti-TNT antibody, with the largest value corresponding to the highest affinity. It is important to notice that the relative affinity values calculated in this study refer to the ability of each molecule to displace anti-TNT antibody specifically from TNB. Thus, as shown in Table 3, the relative affinity values (TNT>TNB>DNT) are consistent with the experimental results obtained.

Known the values of Δf_{max}, K_A and k_A, the ODE describing the change of Δf as a function of time (Equation 5) can be analyzed in Matlab (Figure 6). The detection of TNT in a solution at 10 μg/mL was simulated and observed to fit the experimental data accurately during the initial phase of displacement. Similar results were obtained for simulated and experimental data using lower TNT concentration (1μg/mL, data not shown). The model was therefore considered acceptable and extended to the analysis of TNB and DNT at high concentrations (Figure S-3 and S-4). The mathematical model developed allows establishing a standard curve, which can be used to estimate the concentration of unknown TNT solutions.

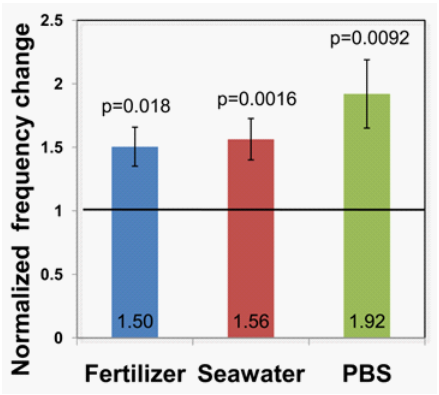


Figure 5: Detection of TNT in dirty environments. The limit of detection of TNT in PBS (green), fertilizer (blue), and seawater (red) was 0.1ng/mL. The black line represents the value of control sample (solutions without TNT). The data analysis was conducted as described in the text (Equation 2), and data points were obtained from at least three independent experiments.

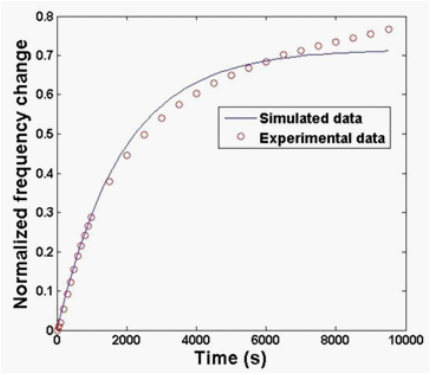


Figure 6: Mathematical model of TNT (10μg/mL) detection. The solid line represents the simulated data obtained from Equation 5, while the open circles are experimental data points obtained from QCM.

Parameter	Δf_{max}	K_A(mL/μg)
TNT	0.73±0.05	5.09±0.61
TNB	0.66±0.04	1.44±0.62
DNT	0.24±0.11	0.47±0.14

Table 3: Parameters obtained from the mathematical model.

Conclusion

We have developed a rapid and accurate QCM based displacement assay for the detection of TNT in liquid phase, by exploiting the cross reactivity of an anti-TNT antibody (A1.1.1) for TNT analogs. In this study, ELISA was first used to evaluate the displacement assay for the detection of TNT, and calculate the relative affinity of the antibody for TNT and selected TNT analogs and the limit of detection of TNT (1ng/mL). Results obtained were comparable to previously reported data. The displacement principle was subsequently used to develop a QCM based assay for the detection of TNT with higher sensitivity and specificity. The limit of detection obtained was an order of magnitude lower than previously reported (0.1 ng/mL). The robustness of this QCM based TNT sensor was confirmed by evaluating TNT detection in dirty environments, such as fertilizer and seawater, and the limit of detection achieved was comparable to that measured in pure TNT solution. As described in the Introduction, fluorescence spectrophotometry has been widely used for TNT detection. This method, however, exhibits low specificity compared to the displacement assay-based QCM sensor reported herein. It was previously demonstrated that fluorescence spectrophotometry can be used to distinguish TNT from nitrobenzene (NB) and DNT because of higher electron deficiency of TNT [17]. However, the specificity of this method is likely to be significantly lower when used to detect TNT in a mixture containing molecules with similar electron transfer properties, such as TNB. In summary, the QCM based displacement assay developed in this study allows detecting TNT at a concentration as low as 0.1 ng/mL within 10 minutes, even in dirty environments containing structurally similar molecules. The kinetics of detection can be predicted using the model described, based on which a standard curve can be established and facilitate on-site detection.

The assay described is based on the competition between two immunochemical interactions: the binding TNB-antibody and the binding TNT-antibody. Therefore, the extent of antibody displacement depends on the ratio between the relative binding affinities of TNT and TNB for the antibody. The limit of detection could be further increased by using a TNT analog with lower affinity than TNB as surface competitor. On the other hand, using a competitor with high affinity is likely to increase the specificity of the assay for detection of TNT in dirty environments.

The use of protein engineering technologies for the selection of an anti-TNT antibody with enhanced affinity for TNT or lowered cross-reactivity with TNT analogs would also allow further enhancing the sensitivity and specificity of this detection method. Finally, we anticipate that the optimized displacement method (coupled with QCM) and model for TNT detection can be modified for the detection of other molecules of interest (e.g. imazethapyr [49] or cocaine [50]), provided that an antibody specific for the molecule of interest, and displaying cross reactivity for analogous molecules that typically limit its detection, has been isolated or engineered.

Acknowledgement

This work is partially supported by the Lockheed Martin Advanced Nanotechnology Center of Excellence (LANCER) at Rice University.

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