A DROPLET-BASED LAB-ON-A-CHIP FOR COLORIMETRIC DETECTION OF NITROAROMATIC EXPLOSIVES

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ABSTRACT

Portable and automated field screening equipment would be very effective in detecting and quantifying explosives at various sites. A droplet-based microfluidic lab-on-a-chip utilizing electrowetting is presented for the colorimetric detection of TNT (trinitrotoluene). The method uses the reaction between nitroaromatics and a strong base which forms the highly colored *Jackson-Meisenheimer* complex. Microliter-sized droplets of TNT are programmed to transport, mix, and react with potassium hydroxide (KOH) on the microfluidic chip. Colorimetric reactions of TNT are characterized both on a spectrophotometer and on the microfluidic chip. The detection of TNT on the chip is linear in the range of 4–20μg/mL with a time-to-result of 2.5minutes. It is also observed that the absorbance peaks of DNT (dinitrotoluene) and TNT are mutually independent and that the presence of DNT does not affect the detection of TNT. Electrowetting also does not seem to influence the colorimetric complex as observed from a comparison of results between a spectrophotometer and on-chip.

1. INTRODUCTION

Recent thrusts in counterterrorism efforts have led to an increased interest in technologies for the detection of explosives. Explosives need to be detected in a variety of complex environments such as mail, luggage, urban areas of high population density (such as airports, subways, stadiums), soils (from munitions manufacturing sites, military bases, land mine fields, post-blast sites), and water (ground, surface, and sea).

Nitroaromatics (TNT, DNT, DNB) form one of the most commonly used class of explosive compounds, with TNT being the most widely used explosive [1]. TNT poses a health risk to humans even at very low parts per billion concentrations in ground water [2] and is suspected to be a carcinogen besides being highly toxic for humans, plants, and animals [3].

TNT is currently analyzed in the laboratory using the high performance liquid chromatography-based U.S. EPA SW-846 Method 8330 [4]. Several commercial field screening test kits are also available for the detection of TNT, based either on immunoassays [5][6] or colorimetric methods [7][8]. In a comparison of all on-site TNT analysis methods, the colorimetric method as detailed in EPA Method 8515 was found to be the most suitable for on-site detection based on accuracy, detection limits, precision, ease of use, cost per sample, and the ability to detect classes of explosives [9]. The EPA 8515 method is based on the reaction between a nitroaromatic and a strong base which forms the highly colored Jackson-Meisenheimer anion, and has a detection limit of parts-per-million.

Current laboratory methods for trace analysis of nitroaromatics have long turnaround times (3 days), are expensive ($1000) and require skilled technicians [10]. Commercial field screening test kits have a shorter turnaround time but still require a skilled on-site technician. There is therefore a need for on-site explosive detection systems that would be fully-automated in addition to being inexpensive, sensitive, reliable, and compatible with a broad range of samples. The advent of microfluidic lab-on-a-chip technology offers such detection systems due to the advantages in portability, reduction of the volumes of the sample and reagents, faster analysis, increased automation, mass manufacturability, and high throughput.

Most microfluidic devices are based on continuous flow which are rigid in design and have limited reconfigurability and scalability in architecture. An alternative approach, which offers a more generic microfluidic platform, is to manipulate the liquid as unit-sized discrete microdroplets. Electrowetting is one of several techniques that have been successfully used to implement a digital microfluidic lab-on-a-chip. Electrowetting refers to the modulation of interfacial tension between a conducting liquid and a solid electrode, by the application of an electric field between them. We have previously utilized electrowetting to demonstrate a fully integrated and automated colorimetric assay for glucose [11]. In this paper, while sample extraction is still manual, we extend the utility of our electrowetting platform by automating the chemical analysis and detection of droplets containing nitroaromatic explosives. The proposed digital microfluidic platform for the detection of TNT is shown in Figure 1. This platform could be used to perform simultaneous optical detection on multiple droplets.

2. MATERIALS AND METHODS

2.1 Chemicals

Commercial grade 2,4,6-trinitrotoluene (TNT) and pure 2,4-dinitrotoluene were obtained from Sandia National Labs. KOH and DMSO were reagent grade. 20 μg/mL stock solutions of TNT and DNT were freshly prepared in DMSO and diluted to obtain various sample concentrations between 4–20μg/mL. Since KOH does not dissolve readily in DMSO, a 0.36M KOH solution was first prepared in DI water and then diluted 2000 times with DMSO to obtain a stock concentration of 180μM. The

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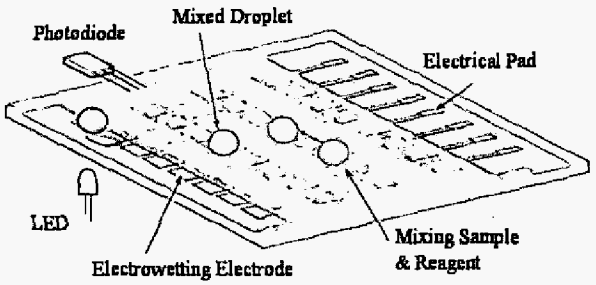

Figure 1- Schematic of the electrowetting lab-on-a-chip integrated with optical detection.

KOH stock solution finally ended up with less than 0.05% water.

2.2 Electrowetting setup and Chip Fabrication

The electrowetting system consists of two parallel electrode plates, a continuous ground plate on top and an addressable electrode array (pitch = L) on the bottom plate as shown in Figure 1. A spacer separates the top and bottom plates, yielding a fixed gap (H). The droplet is sandwiched between the two plates, and surrounded by immiscible silicone oil, which prevents evaporation of the droplets and also reduces the voltages required for transporting the droplets. The electrode array is insulated (Parylene C) from the liquid and both surfaces are hydrophobized (Teflon AF). Indium Tin Oxide (ITO), a transparent conductor, is used as the material for the electrodes, to enable easy integration of optical detection methods. In the experiments reported in this paper, we have used electrowetting chips with an electrode pitch (L) of 1.5 mm and a gap spacing (H) of 600 μm.

2.3 Colorimetric method for the detection of TNT

Nitroaromatic compounds such as TNT/DNT react with nucleophiles (bases) such as hydroxides and alkoxides, to form colored *Jackson-Meisenheimer* complexes. Acetone, acetonitrile and methanol have been the most popular choices for TNT analysis even though this reaction has been demonstrated in various organic solvents. These solvents are miscible with silicone oil in the current setup. DMSO is another versatile solvent which dissolves most aromatic hydrocarbons, including nitroaromatics such as TNT, and yet is immiscible with silicone oil, making it a compatible solvent with our current setup. Also DMSO is completely miscible with water in all proportions and has a low order of toxicity for use in the field. For these reasons we chose to use DMSO as the solvent to develop the TNT reactions. DMSO is also known to enhance the stability of the *Jackson-Meisenheimer* complex [12]. In the experiments reported in this paper, potassium hydroxide (KOH) was used as the base to react with TNT to produce the colored complex. To the best of the authors' knowledge the reaction between TNT/DNT and KOH using DMSO as the solvent has not been characterized in terms of the absorbance peaks of the colored complexes, and is therefore reported in this paper.

2.4 Optical Detection

The colored *Jackson-Meisenheimer* complex is detected using a simple absorbance measurement system comprising of an LED (505nm) and a photodiode, perpendicular to the plane of the electrowetting chip, as shown in Figure 1. The absorbance is calculated from the equation $A=\ln(V/V_o)$, where V is the photodiode voltage output using TNT as the sample and V_o corresponds to the blank absorbance.

2.5 System Operation Protocol

The reactions were performed on the chip in three steps: dispensing, electrowetting-enabled mixing, and colorimetric detection. Droplets of TNT and KOH are dispensed manually by a pipette on the electrowetting chip. These two droplets are merged by applying voltages to the appropriate electrodes. The merged droplet is further mixed by shuttling it across four electrodes for 30 seconds at an actuation voltage of 50 V. Our earlier results indicate that mixing should be complete in less than 30 seconds for this pattern of mixing [13]. At the completion of mixing, the absorbance is measured using the LED (light emitting diode)/photodiode setup described earlier. All the reactions were performed at room temperature.

3. Results and Discussion

3.1 Absorbance spectra of colored product

The absorbance spectrum of the colored product resulting from the reaction between TNT/DNT and KOH, in DMSO is shown in Figure 2. TNT has absorbance peaks (λ_{max}) at 336 nm and 510 nm, while DNT has peaks at 407 nm and 657 nm. It should also be noted that TNT has absorbance minima close to the absorbance peaks of DNT and vice-versa. The TNT reaction was monitored at 505 nm (close to λ_{max}=510nm), which is the peak emission wavelength of the LED used in the on-chip optical measurements.

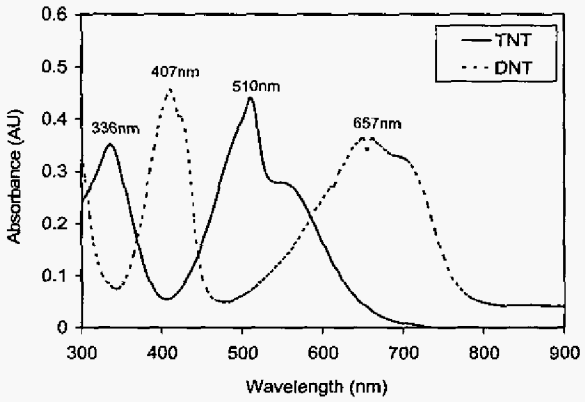

Figure 2 - Absorbance spectrum of colored complexes formed in the reaction between TNT/DNT and KOH, using DMSO as the solvent.

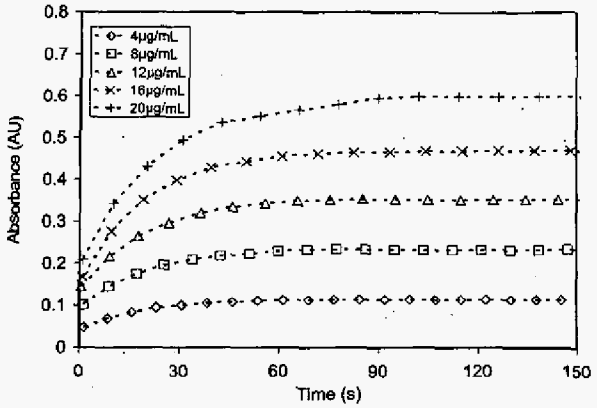

Figure 3 - Absorbance of the colored complex for various concentrations of TNT as a function of time on the spectrophotometer.

3.2 TNT reaction on a spectrophotometer

The TNT reaction was initially characterized on a bench top spectrophotometer (Gensys 20). 500µL of 180µM KOH was added to 500µL of the sample (4-20µg/mL) at T=0s and the absorbance was monitored at 505nm on the spectrophotometer. Figure 3 shows the absorbance of the colored complex, as a function of time. From the figure note that the absorbance remains stable after T=120s. Figure 4 plots the absorbance obtained at T=150s (after stable color is reached) as a function of the TNT concentration. The absorbance is linear up to 20µg/mL which was the highest concentration tested, with an R^2 (measure of goodness of fit) of 99.94%.

3.3 Detection of TNT on-chip

A 1.5µL droplet of the TNT sample (4-20µg/mL) and a 1.5µL droplet of 180µM KOH was dispensed and mixed to react on the chip. The blank absorbance is measured with the KOH droplet. Figure 5 shows absorbance under the reaction conditions as a function of time. T=0s corresponds to the time instant at which the droplets were merged. From the figure it can be observed that the color stabilizes around T=150s. The absorbance measurements are therefore taken 150s after the droplets were merged.

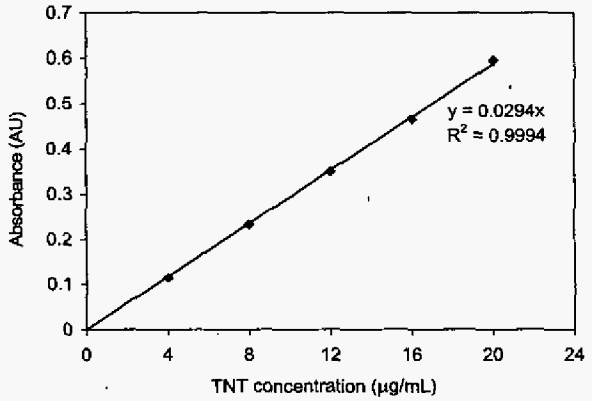

Figure 4 - Calibration curve of absorbance with respect to the concentration of TNT demonstrating a linear relation on the spectrophotometer.

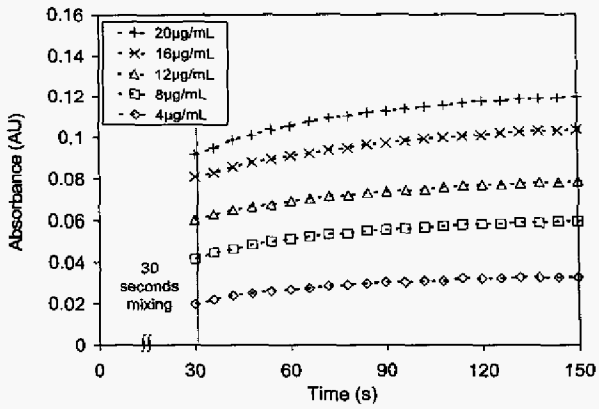

Figure 5 - Absorbance of the colored complex for various concentrations of TNT as a function of time on the electrowetting chip.

A comparison of reaction kinetics after 30s in Figure 3 and Figure 5 shows that qualitatively the trends are similar. On-chip reaction kinetics data are not available before 30s because the droplets are being shuttled for mixing during that time. It should be noted that the absorbance values are not the same, due to the different optical path lengths for each case. From Figure 6, the absorbance is linearly related to TNT with an R^2 of 98.63% up to 20µg/mL (the highest concentration tested).

3.4 Detection of TNT in a mixture of TNT and DNT

From Figure 2 we can see that the absorbance spectra for TNT and DNT are mutually independent. This means that TNT can be quantitatively estimated even in the presence of DNT. To verify this hypothesis, mixtures of TNT and DNT were analyzed in various ratios on a spectrophotometer at 505nm (absorbance peak of TNT), and the results are shown in Figure 7. 100% TNT or DNT corresponds to a concentration of 20 µg/mL. From Figure 7, the absorbance at 505 nm is linear with varying concentrations of TNT in the TNT+DNT mixture and is negligible for 100% DNT and 0% TNT. Therefore, using this colorimetric reaction, TNT can be detected even in the presence of DNT.

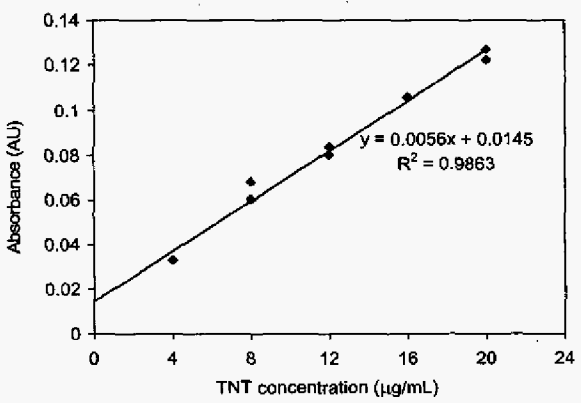

Figure 6 - Calibration curve of absorbance with respect to the concentration of TNT demonstrating a linear relation on the electrowetting chip.

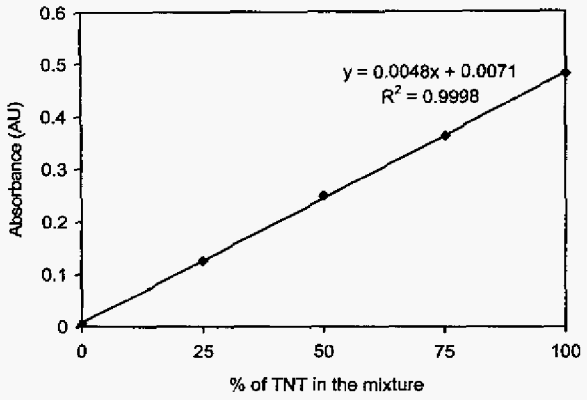

Figure 7 - Absorbance of TNT at 505 nm with varying amounts of TNT+DNT demonstrating the non-interference of DNT with the TNT reaction. At 0% TNT in the mixture, DNT is 100%.

4. CONCLUSIONS

A digital microfluidic lab-on-a-chip based on electrowetting actuation of droplets has been demonstrated for the detection of 2,4,6-trinitrotoluene (TNT) in DMSO. The colorimetric detection is based on the absorption of the *Jackson-Meisenheimer* complex formed as a result of the reaction between the nitroaromatics and a strong base. We have demonstrated a linear range of detection for TNT between 4μg/mL–20μg/mL and the time for each reaction-to-detection is about 2.5 minutes. The lab-on-a-chip can handle multiple samples in parallel, so the detection time would still be 2.5 minutes for simultaneous detection of a number of samples. Advantages of such a lab-on-a-chip system include complete automation and integration, on-chip calibration, nanoliter sample/reagent consumption, increased parallelism, and portability. Since the current lab-on-a-chip platform is being developed as a generic analytical platform, analysis of a broader range of samples encompassing both chemical and biological realms can be integrated on the same platform.

Future work would involve improving the detection limits of the system from μg/mL to ng/mL. Also, experiments need to be performed on nitroaromatic samples extracted from soil and post-blast residues to establish the suitability of a digital microfluidic system for field screening of nitroaromatics. Methods will also be adapted for the colorimetric detection of nitramines and nitrate esters. The system will also need to work with a wider range of solvents including acetone and acetonitrile, which are more commonly used solvents. Even though we have automated analysis, extraction is still manual therefore extraction procedures need to be automated in order to have a fully automated system.

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