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Lab on a Chip Sensor Platform for Explosives and CBW Toxin Detection

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Abstract *– There has been significant interest in the adaptation of lab on a chip devices for the separation and detection of chemical and biological toxins. Potential toxic and/or hazardous analytes of concern in our program include BWA protein toxins,e.g., SEB and ricin, ingestible CW toxins, e.g. alkaloids and rat poisons, and nitroaromatic explosives indicative of IED's. Discussion will center around our efforts to enhance sensitivity and selectivity on a microchip by incorporation of micro-solid-phase extraction, microchip bubble-cell long pathlength UV detection,organically-modified sol-gel materials for electrochromatography, protein recognition aptamers, and microfluidic-based displacement immunoassays.*

Keywords *– lab on a chip, capillary electrophoresis, antibody, aptamer, ricin, explosives, staphylococcus entertoxin B, microchip, displacement immunoassay, alkaloids*

I. INTRODUCTION

Concerns associated with the potential for future terrorist attacks have prompted investigations into novel methods for sensitively detecting a wide range of toxic analytes. Potential toxic and/or hazardous analytes of concern in our program include biowarfare agent protein toxins, e.g., Staphylococcus enterotoxin B (SEB) and ricin, ingestible chemical toxins, e.g. alkaloids and rat poisons (e.g., sodium monofluoroacetate, MFA), and nitroaromatic explosives indicative of improvised explosive devices. Due to the many advantages associated with lab on a chip microdevices, including portability, minimal waste generation, rapid separation times, small sample size requirements, ease of integration and low cost, there has been significant interest in investigating the adaptation of this platform to the separation and detection of this broad range of toxins. Discussions here will center around our efforts to enhance sensitivity and selectivity on a lab on a chip microdevice by incorporation of micro-solid phase extraction (μ-SPE), bubble cell long pathlength UV detection on a microchip, organically modified sol gel materials for electrochromatography, protein recognition aptamers, and microfluidic based displacement immunoassays.

II. PROPOSED APPROACH

Microchip-based capillary electrochromatography of nitroaromatic and nitramine explosives was explored using sol-gel stationary phases deposited directly in a quartz microchip device.[1] Unique to this work, is the exclusive use of alkylated-trimethoxysilane precursors in the gel solution.[2] Using alkylated precursors allows for the synthesis of a hydrophobic stationary phase in a single step. Three sol-gel formulations of increasing hydrophobicity and suitable for the separation of explosives are established from methyl- and ethyl-trimethoxysilane precursors.

The feasibility of utilizing direct detection for small toxic molecules, such as monofluoracetic acid (MFA), is examined using the capillary electrophoresis microchip coupled with a contactless conductivity detector. Conductivity detection is based on the same physical property that dictates electrophoretic separations, electrophoretic mobility. The main advantages of this detection mode are its universality and simplicity. For contactless conductivity detection, small molecular weight analytes, such as MFA, no longer must be UV-absorbing or derivatized, but simply ionic in form in a chosen separation buffer. The detector consists of inexpensive electronics and metal electrodes.[3] The contactless nature of the electrodes outside of the solution simplifies the alignment and positioning of the detection cell and prevents fouling of electrodes.

The general chemical properties of chemically toxic alkaloids, however, rule out their detection by other more commonly utilized detection methods on a microchip, including conductivity, fluorescence or electrochemistry. Despite the wide applicability of absorbance detection and more than a decade of research since the first introduction of the lab on a chip platform, there are startling few examples of absorbance detection on capillary electrophoresis (CE) microchip devices. The predominant reason for this is their inherently short pathlengths, and the resulting poor sensitivity. To promote sensitivity, we have investigated two alternate approaches: 1) microchip bubble cells,[4] and 2) three dimensional microdevice designs, both of which promote longer pathlength cells for UV detection in quartz microchips

Protein recognition elements have been applied to the microchip to enable more selective detection of target protein toxins. Aptamers are single stranded DNA/RNA molecules that have been selected from synthetic nucleic acid libraries to exhibit binding constants for target species that rival and/or exceed the specificity of antibodies.[5] We have utilized capillary electrophoresis to investigate the interaction of the protein toxin, ricin, with a fluorescently tagged RNA aptamer which has been shown to be responsive to the A chain of ricin.[6] Alternatively, a microchip-based, displacement immunoassay was utilized for investigating the competitive

interaction of fluorescently labeled and unlabelled staphylococcal enterotoxin B (SEB) with antibody functionalized microbeads. The glass microchip device consisted of a microchannel that contained a double weir structure for supporting the microbeads.[7]

III. RESULTS

Microchip-based CEC of nitroaromatic and nitramine explosives with UV absorbance detection is described. The stationary phase was deposited in the microchip using the sol-gel process. Increasing alkyl-chain length improved resolution significantly, allowing for the separation of up to seven explosives. Direct injection onto the head of the stationary phase for long injection times, results in sub- mg l^{-1} detection limits with little effect on separation efficiency.

Figure 1 illustrates the Y-configured microchip utilized in this work. A close-up image of the electro-chromatographic sol gel stationary phase deposited in the microchannel is indicated as an inset. The hydrophobicity of the sol gel phase enables the solid phase to perform solid phase extraction or preconcentration of explosives onto the head of the column, in addition to high resolution separation following conversion of the buffer to an organic eluant.

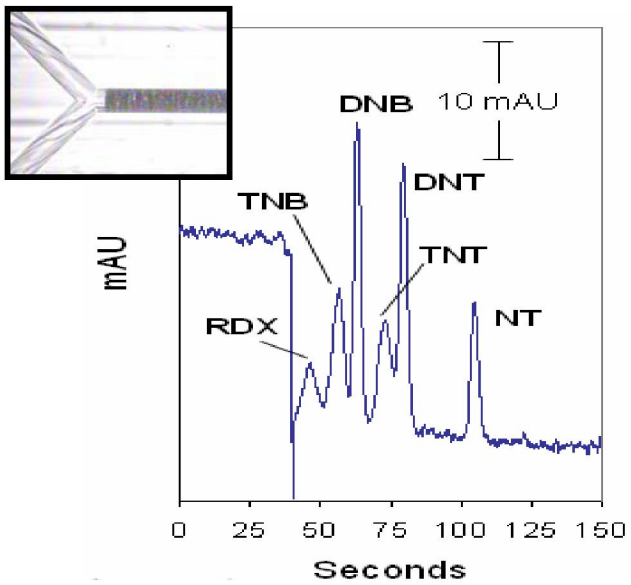

Figure 1: Microchip electrochromatographic separation of 500 ppb sample of explosives on 10% C2 sol gel column. Inset is a photograph of the Y-configured microchip with sol gel deposited in microchannel.

The separation and UV absorbance detection of four toxic alkaloids, colchicine, thiocolchicine, colchicoside, and thiocolchicoside, on a microchip-based capillary electrophoresis device are reported. To increase the sensitivity of UV absorbance detection, optical cells with extended pathlength are integrated into the separation channel during the microfabrication process (Figure 2). The absorbance values realized on the microchip using these optical cells were proportional to the increase in average depths according to the Beer-Lambert Law, resulting in sensitivity enhancements by as much as five times. Linearity of response was observed from 5.0 mg/L to 500 mg/L of colchicine, with detection limits ranging from 2-6 mg/L depending upon the specific alkaloid and the dimension of the optical cell. The extraction of colchicine from spiked milk samples was performed and an average recovery rate of 83% with a relative standard deviation of 3.8% was determined using the optimized conditions on the microchip.

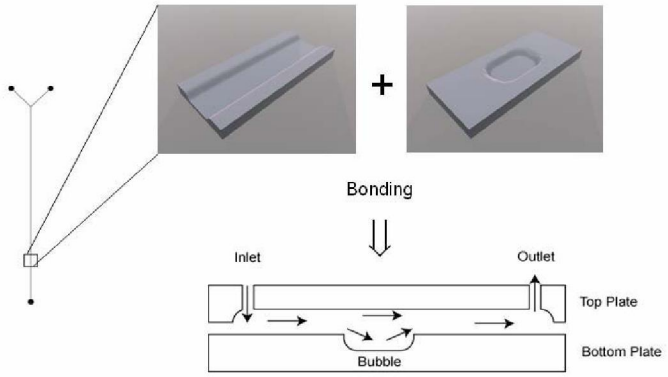

Figure 2: Microchip bubble cell for UV absorbance detection.

Because of the chemical similarity of MFA to citric acid, a primary component of juice, a citrate/histidine buffer was chosen to prevent the contactless conductivity detector from being overwhelmed by the ionic components evident within a real sample. By bringing the pH down to 3.5, acetic acid, for example, is no longer ionized due to the nature of its pKa, thereby, masking its presence very effectively. Attempts to remove and preconcentrate MFA from real samples such as orange juice through the use of solid phase extraction have proven unsuccessful. Direct dilution of MFA contaminated juice samples by a factor of ten has enabled excellent quantitative results, despite the sacrifice in sensitivity due to dilution. Detection limits when taking into account dilution effects are ~1-2 mg/L for the detection of MFA in orange juice. Based on the reported monofluoroacetate LD_{50} value (714 μg/kg body mass, human male, oral), these low mg l^{-1} detection limits are adequate in protecting the public from the possible contamination of fruit juices by MFA. For a small toddler weighing 10 kg, for example, the volume of fruit juice that would have to be consumed in order to attain a toxic level, assuming a contamination level of 2 mg l^{-1} MFA that is at our limit of detection capability, would be a one-time consumption of 3.57 liters. We have now examined additional juice matrices, including apple and cranberry, as

well as tap water. In all cases, detection limits better than orange juice were obtained.

The ability to detect subnanomolar concentrations of ricin using fluorescently tagged RNA aptamers is demonstrated. Aptamers rival the specificity of antibodies and have the power to simplify immunoassays using capillary electrophoresis. Under non-equilibrium conditions, a dissociation constant, K_d, of 134 nM has been monitored between the RNA aptamer and ricin A-chain. Using this free-solution assay, the detection of 500 pM (~14 ng/mL) or 7.1 attomoles of ricin is demonstrated. The presence of interfering proteins such as bovine serum albumin and casein do not inhibit this interaction at subnanomolar concentrations. When spiked with RNAse A, ricin can still be detected down to 1 nM concentrations despite severe aptamer degradation. This approach offers a promising method for the rapid, selective, and sensitive detection of biowarfare agents.

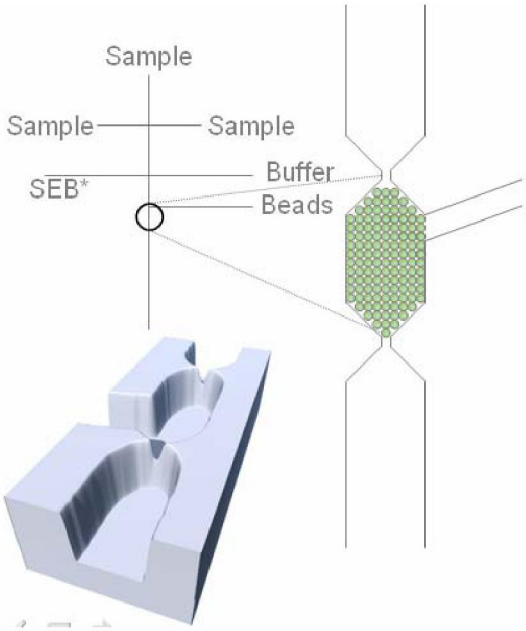

Figure 3: Microchip weir design for performing displacement immunoassay for SEB.

A microchip-based, displacement immunoassay for the sensitive laser-induced fluorescence detection of staphylococcal enterotoxin B (SEB) is presented. Shown in Figure 3 is the microchip utilized to contain the SEB antibody labeled beads. The microchip design contains a narrow weir through which fluid flows, but the beads are contained behind. The displacement assay was performed without user intervention and produced quantitative results in 20 minutes. Linear detection responses were observed over 6 orders of magnitude and provided detection limits down to 1 fM (28.5 fg/mL). The surprisingly low detection limits are hypothesized to arise from field-based enrichment analogous to field-amplified stacking, chromatographic effects, and limited diffusion lengths in the microbead bed. The assay was challenged with bovine serum albumin, casein, and milk sample matrices. This system has the potential to provide highly sensitive detection capabilities for target biomolecules.

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