ACS SENSORS

Letter

Cite This: ACS Sens. 2020, 5, 13−18

pubs.acs.org/acssensors

Air2Liquid Method for Selective, Sensitive Detection of Gas-Phase Organophosphates

Michael C. Brothers,[†,‡] Sungbong Kim,[§,∥] Doug Adkins,[⊥] Pat Lewis,[⊥] Joshua E. Smith,[#] Diana Ostojich,[¶] Steve S. Kim,[†] John A. Rogers,[¶,□,●,△,▼,⬢] and H. Mitchell Rubenstein*[,†]

[†]711th Human Performance Wing, Air Force Research Laboratory, Wright-Patterson AFB, Ohio 45433, United States

[‡]UES Inc., Dayton Ohio 45432, United States

[§]Department of Materials Science and Engineering and [∥]Frederick Seitz Materials Research Laboratory, University of Illinois at Urbana−Champaign, Urbana, Illinois 61801 United States

[⊥]Defiant Technologies, Albuquerque, New Mexico 87109, United States

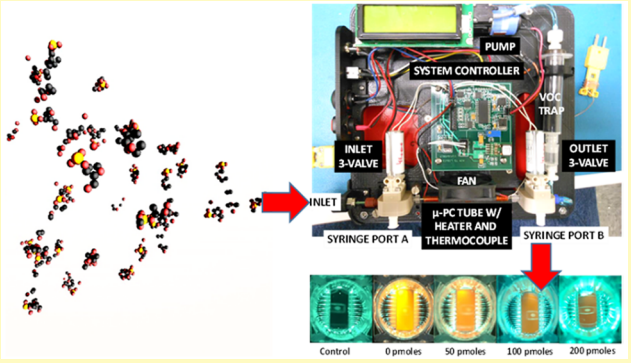
[#]Department of Chemistry, Alvernia University, Reading, Pennsylvania 19607, United States

[¶]Department of Materials Science and Engineering, [●]Center for Bio-Integrated Electronics, [△]Department of Biomedical Engineering, and [▼]Department of Chemistry, Northwestern University, Evanston, Illinois 60208, United States

[□]Simpson Querrey Institute and [⬢]Department of Neurological Surgery, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, United States

Supporting Information

ABSTRACT: Environmental hazards typically are encountered in the gaseous phase; however, selective sensing modalities for identifying and quantitating compounds of interest in an inexpensive, pseudo-real-time format are severely lacking. Here, we present a novel proof-of-concept that combines an Air2Liquid sampler in conjunction with an oil-in-water microfluidic assay for detection of organophosphates. We believe this proof-of-concept will enable development of a new platform technology for semivolatile detection that we have demonstrated to detect 50 pmoles (2 ppb) of neurotoxic organophosphates.



KEYWORDS: *gas sensor, semi-volatile detection, organophosphate, microfluidic assay, selective sensor*

Detection of low levels of organophosphates (OPs) in the gaseous phase is of high relevance to maintainers, especially in the airline industry, due to their presence in common engine oils and lubricants and their high toxicity.[1] OPs inhibit the enzyme acetylcholine esterase (AChE), preventing hydrolysis of acetylcholine (ACh),[2] causing over-activation of the parasympathetic nervous system. This results in bronchoconstriction, hypotension, and prolonged muscle contractions.

However, specific identification and detection of hazardous semivolatile compounds (sVOCs) at relevant levels in industrial settings, including OPs, is challenging due to their high toxicity (Permissible Exposure Limits (PEL) of 1 ppm or less for OPs[3]), their low chemical reactivity, and their presence in complex mixtures containing background volatile organic solvents (VOCs), such as gasoline and diesel fuels. In order to identify and quantify low concentrations of sVOCs, multiple mechanisms can be deployed: (1) deployment of sensitive detection techniques, (2) preconcentration of air samples, and/or (3) assay technologies for signal amplification. Standard high-sensitivity detection systems, such as photoionization detectors (PIDs), are cross-reactive and/or can be confounded by background contaminants, preventing target identification without incorporating chemical separation or orthogonal information.[4−7] Since these sensors are often deployed in environments where common interfering compounds are present (i.e., isopropyl alcohol, toluene, hexanes) that have PELs > 100 ppm, >100× higher than toxic compounds of interest, even techniques that can fingerprint the environment, such as ion-mobility spectrometry (IMS) and surface acoustic wave (SAW) lack the resolution needed to identify and discriminate acetylcholinesterase inhibitors.[5−7] Preconcentrators using hydrophobic sorbents followed by thermal desorption have been demonstrated for sVOCs using GC-MS,[8] GC-PID,[9] and GC-SAW instruments, where the temperature at which the compound is eluted provides

Received: August 21, 2019
Accepted: December 13, 2019
Published: December 13, 2019

DOI: 10.1021/acssensors.9b01624
ACS Sens. 2020, 5, 13−18

information toward identification. However, both PIDs and SAWs lack the absolute specificity for the analyte of interest, leading to potential false positives. Additionally, SAWs are known to be semiselective at best and prone to degradation based on the analytes and background chemicals present.[7] In comparison, GC-MS, while quantitative and highly specific, is difficult to deploy in the field. Typically, miniaturized GC-MS units are costly (>$50,000 per unit), large (>20 lbs), and require technical expertise for optimization, maintenance, and troubleshooting.

Biochemical assays are selective and sensitive, but require transition to the aqueous phase, often require significant manual labor, and typically take hours to complete. Substantial progress has been made on developing microfluidic biochemical assays that measure AChE inhibition to determine the concentration of OPs; however, the devices as currently formatted are only applicable to aqueous phase OPs.[10] In comparison, for one exemplary case, tricresyl phosphate (TCP) was collected from the gaseous phase onto an alumina column, followed by hydrolysis and direct detection of the cresol group using chronoamperometry.[11] Chronoamperometry detected as low as 750 pmol of cresol, corresponding to 250 pmol of TCP. However, this approach only applies to redox-active byproducts, such as the cresol phosphates produced after hydrolysis of TCP. Additionally, the method requires conversion of the sparingly soluble OPs into soluble, redox-active cresols. Therefore, additional methods must be explored to detect more chemically diverse gas-phase analytes, including OPs, in the aqueous phase.

Aqueous phase assays have been developed and deployed for a diverse array of analytes. These assays produce reporter molecules, such as a redox active product, or a product that can be sensed optically. Often, chemical or biochemical reactions are coupled together, where the product of one reaction provides the substrate for the subsequent reaction to eventually produce the reporter molecule. Commonly used aqueous phase assays include (1) chemical reactions that result in the generation of reporter molecules, (2) enzyme catalyzed reactions that result in the analyte or a competitive substrate generating reporter molecules,[12] and/or (3) enzyme-linked immunoassays (ELISAs)[13] that generate reporter molecules following immunoprecipitation.

AChE activity, as well as inhibition (i.e., by OPs and nerve agents[2]) has been detected using enzymatic catalyzed reactions. In short, byproducts from AChE in the presence of its substrates (ACh or acetylthiocholine) are converted through a series of enzymatic reactions to reporter molecules. However, the poor solubility of OPs (SI Table 1) in aqueous media has to-date limited the transfer of collected OPs to AChE, limiting the efficacy of aqueous assays. Previous work has shown the efficacy and robustness of AChE enzymes in highly hydrophobic solvents (logP > 2).[14] It is hypothesized that enzymes maintain their activity through retention of a water shell surrounding the enzyme,[15] enabling the enzyme to retain its structure. Thus, introduction of oil-in-water microfluidics enables (1) retention of the aqueous-phase shell, (2) transfer of the hydrophobic OPs captured on the sol−gel to the AChE, and (3) delivery of reagents required to produce reporter molecules, correlating to enzymatic activity.

In this manuscript, we perform a proof-of-concept study to demonstrate the efficacy of microfluidic assays for compounds collected in the gaseous state using a preconcentrator (PC). More specifically, we integrate a sVOC gas collection device that also serves as a PC with a microfluidic device for highly sensitive detection of AChE inhibitors; these components can be fully integrated and automated into a prototype device. In the future, we envision this method and these devices can be modified to detect a variety of hydrophobic, gaseous analytes.

METHODS

Gas-to-Liquid TBP Collection. Samples of tributyl phosphate (TBP) were collected using a gas-to-liquid extractor (Figure 1) by sampling the headspace of a beaker containing ∼10 mL TBP. Air was sampled into an inlet, and routed using a check valve on both the inlet and outlet of a micro preconcentrator (μ-PC) trap using a Virtual Industries (VI) pump at 60 mL/min.

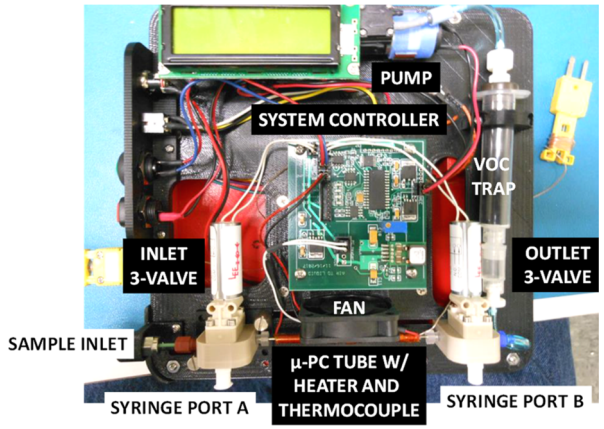


Figure 1. Integrated μ-PC air sampling device with ports for air sampling, solvent collection, and regeneration.

Fabrication of μ-PC. The μ-PC is a 1/16" ID stainless steel tube that is wrapped with a heater for removal of residual solvents. The tube contains 98-mg of 100-μm-diameter glass beads coated with a proprietary Xerogel by Defiant Technologies (Albuquerque, NM).

Design of Integrated Device for Semi-Automated Sampling. Two Neptune Research 3-way valves were implemented before and after the μ-PC to enable both air sampling and fluid washing. When both check valves were energized, air is collected from the environment through a 30 mil sample inlet tube made from Sulfinert stainless steel tubing through the 3-way valve to the μ-PC. The flow path then continued through the second 3-way valve, through both a fluid trap and a VOC trap (activated carbon), and finally through the pump (VI). After collection, the check valves are de-energized, enabling flow through two Luer-lock fluidic ports. Hexane was injected via syringe backward through the μ-PC (into the second three-way valve), serving as a back-flush, and transferring hexane into a collection syringe. The residual hexane is removed via air flow and heating of the xerogel to 60 °C for 5 min, thus regenerating the collector.

Microreactor Design and Fabrication. A serpentine microfluidic channel was designed and implemented to both maximize the microfluidic channel length and minimize device size. Initial designs were made from polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) cured at 70 °C for 4 h using cast molding. The mold was prepared using the following steps: (1) creation of a protective mask on the silica wafer by spin-casting KMPR1010 photoresist at 3000 rpm for 30 s and baking on a hot plate at 110 °C for 3 min, (2) patterning of the protective layer using photolithography (UV irradiance for 300 mJ·cm^{-2}, and developing for 2 min with developer MF917), (3) formation of the mold by deep reactive-ion etching of the silica wafer (STS Pegasus ICP-DRIE, SPTS Technologies Ltd., UK) to a depth of 400 μm, (4) surface modification of the mold through spin-casting a layer of poly(methyl methacrylate) (PMMA, 3000 rpm for 30 s, baking at 180 °C for 10 min) to add an anti-adhesion layer, (5) casting and curing of PDMS, forming the μ-

DOI: 10.1021/acssensors.9b01624
ACS Sens. 2020, 5, 13−18

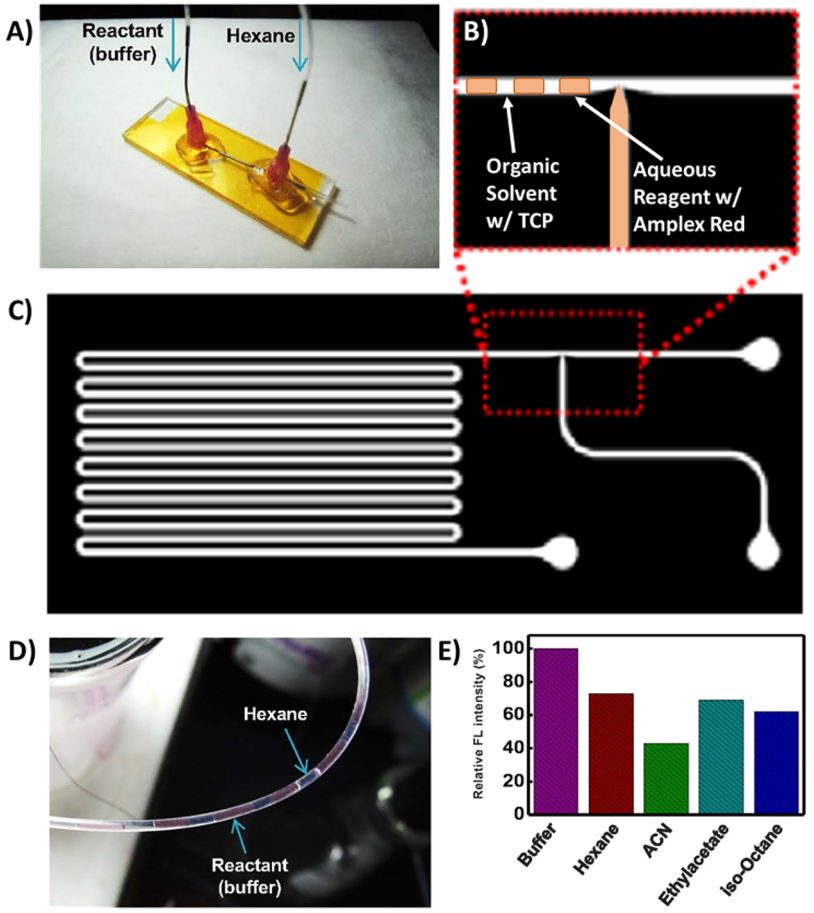


Figure 2. Oil-in-water droplet generation (A, B) enables hexane (clear) and aqueous reagent solution (orange) to create plugs containing OP analyte (hexane) and inject into microreactor (C), that create discrete droplets (D). Testing of various organic solvents through this setup (50 μL/min) demonstrated a smaller loss in AChE activity for less polar solvents including hexane, consistent with literature[14] (E).

channel. The PDMS μ-channel was then annealed to a transparent slab of PDMS (~4 mm thickness) after exposing the surfaces to UV-plasma for 1 min in the presence of oxygen. To prevent deformation by the solvent due to swelling,[16] later devices were fixed and recasted by pouring 10:1 PDMS and curing in a rigid PMMA case (30 mm × 50 mm area and ~15 mm height) which is prepared via 2D laser cutting and epoxy bonding.

Biofunctionalization of Reactor. Based on previous methods,[17] amine groups via (3-aminopropyl) triethoxysilane (3-APTES) were introduced by mixing acetone and 3-APTES at a 7:3 ratio (Sigma-Aldrich, MO), and injected into the PDMS-coated microchannel at a 100 μL/min flow rate at 60 °C for 4 h. After functionalization, a 4% glutaraldehyde solution in phosphate buffered saline (PBS) was injected at ~50 μL/min flow rate for 4 h. Finally, enzymes of AChE (12.5 μL/mL), choline oxidase (COX, 12.5 μL/mL), and horseradish peroxidase (HRP, 5 μL/mL) (~0.04 Unit AChE, ~0.06 Unit COX, and ~0.14 Unit HRP, respectively) were immobilized on the microchannel surface through injection of the protein mixture at a flow rate of 30 μL/min for 2 h. The bioreactor was then filled with PBS and stored at 4 °C. Bradford assays were used to determine the concentration of the enzyme cocktail before (~2 mg/mL) and after functionalization (SI Figure 1) to determine the amount of protein functionalized.

Oil in Water Microfluidics. Oil-in-water droplet flow was generated by the perpendicular merging of two microchannels (~5 to 25 μL/min flow rate for each), one that contained hexane from the Air2Liquid device, and the other that contained the aqueous phase reagent mixture. Figure 2A shows the design of droplet generation (Figure 2B) from an external device (channel width 200 μm and depth 400 μm); other devices used for preliminary data used an internal oil-in-droplet generator (Figure 2C) to simplify the design.

Biochemical Assays in Reactor and Detection. The chain reaction within the microreactor was performed with reactant mixtures of ACh (100 μM) and Amplex Red (5 μL/mL of buffer) as the mixture was injected into the microreactor at 30 °C. Fluorescence from the reaction was obtained by exciting the resorufin fluorophore at ~550 nm (~green), and measuring emission at ~600 nm (~orange).

Eluents were collected in a standard PMMA cuvette, or a custom ~100 μL flow-through aluminum cuvette sealed with PMMA (SI Figure 2B). To perform the fluorescence measurements, an excitation LED was obtained (350-1569-1-ND, Dialight NJ, light source at ~565 nm wavelength), as well as an excitation filter (550BP 180, Omega Optical VT, ~550 nm wavelength), polarizing filter (10CGA-610, Newport Co. CA, barrier filter 610 nm wavelength) and photo detector (PD; 754-1930-1-ND, Kingbright Taiwan) to prevent bleed-over of excitation and emission light. The assembly was covered with black PDMS to eliminate external light. The current from the photodetector was measured using a digital multimeter (DMM, National instruments) which corresponds to fluorescence intensity. The continuous changes in current were recorded and stored digitally. The noise from the recorded data was removed by application of a low pass digital filter (1 Hz) as part of the data processing.

RESULTS AND DISCUSSION

Selection of Enzymatic Assay. Previous work demonstrated the utility of a fluorimetric assay using reactions mediated by AChE, COX, and HRP;[18] these enzymes were immobilized on the surface using APTES/glutaraldehyde

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ACS Sens. 2020, 5, 13−18

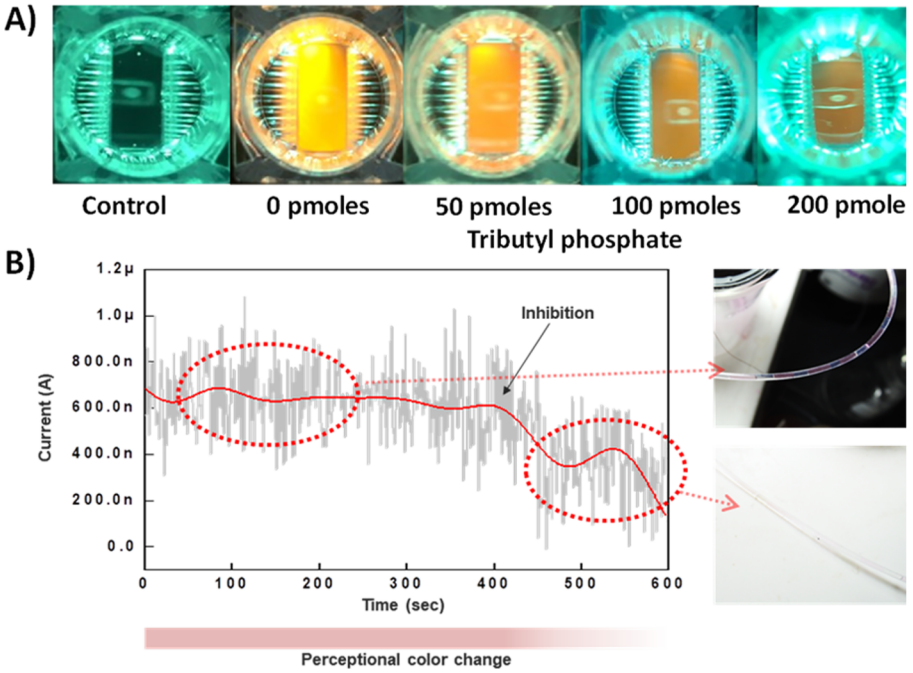

Figure 3. Optimized conditions enabled detection of as little as 50 pmol of TBP after collection in a cuvette (A). A miniaturized, transportable fluorescence detector was then used to detect as low as 200 pmol of TBP (B).

chemistry (see Methods[17]). For this setup, a fluorescent byproduct would be produced only if the AChE remained uninhibited (i.e., no OPs were present) using the following reactions below:

$$\text{Acetylcholine(aq)} \xrightarrow{\text{AChE}} \text{Acetate(aq)} + \text{Choline(aq)} \quad (1)$$

$$\text{Choline(aq)} \xrightarrow{\text{COX}} \text{Betaine Aldehyde(aq)} + H_2O_2\text{(aq)} \quad (2)$$

$$H_2O_2\text{(aq)} + \text{Amplex Red(aq)} \xrightarrow{\text{AChE}} H_2O\text{(l)} + \text{Resorufin(aq)} \quad (3)$$

The final product, resorufin, a fluorescence indicator that at pH = 7, has optimal λ_{ex} = 563 nm and λ_{em} = 587 nm. Thus, fluorescent intensity directly correlates with enzymatic activity. Amplex red, in contrast, has no fluorescent activity. The favorable enzymatic kinetics of AChE (k_{cat} = 1.4 × 10^4) enables a high production of choline per enzyme; minimizing the total number of enzymatic sites increases the sensitivity of the assay, as each site represents a larger percentage of the total choline production. This, coupled with an excess of HRP and ChO (fast steps in chain reaction), results in rapid assessment of AChE activity, producing the fluorimetric output that corresponds to enzyme activity/inhibition. The end result is a system that detects pmol of OPs in less than 1 h due to the rate of choline, and thus resorufin production.

Initial Design Considerations. OPs are known to be highly insoluble in aqueous solutions. Therefore, acetonitrile (ACN) is typically used as the solvent of choice for HPLC assays[19] due to the high solubility of OPs in ACN; we hypothesize that the similar dipole moment (D) of ACN and OPs (3.92 D for ACN, 2.92 D for TBP, and 2.97 D for triphenyl phosphate) explains this observation. Additionally, ACN is highly compatible with PDMS, which is the primary material used in microfluidic devices.

We integrated an enzymatic inhibition assay due to its high level of sensitivity and selectivity. However, ACN has been demonstrated in our system and in the literature[14] to cause irreversible denaturation of proteins due to removal of the solvent shell, causing protein misfolding. More specifically, a previous study[14] tested the change in enzymatic turnover of immobilized AChE post immersion in organic solvents. They determined that while moderately polar solvents caused enzyme misfolding, highly hydrophobic solvents with low water miscibility caused the enzymes to retain a crystalline water shell, enabling high levels of enzymatic activity; similar results were observed in our device as well using an oil-in-water setup (see Methods, Figure 2A–D). As demonstrated, the bioreactor retained greater enzymatic activity when exposed to nonpolar solvents, such as ethyl acetate and hexane, compared to moderately polar solvents (i.e., ACN, Figure 2E).

Preconcentrator Design and Implementation. A Xerogel (μ-PC) was formulated and then coated on microbeads that could withstand organic solvents using proprietary methods (Defiant Technologies). To test the efficacy and saturation points, the headspace over liquid TBP was sampled at a flow rate of 60 mL/min. Doubling the sample time from 5 to 10 min more than doubled the amount of collected TBP; sampling for an addition 15 min did not result in the proportional increase, suggesting that the saturation of the current system exists around ∼20 μg (SI Table 2). For the 10 and 25 min collection steps, 82% and 80%, respectively, of the TBP was released in the first aliquot of 1 mL hexane, demonstrating the high solubility and device compatibility of the chosen solvent. Even more promising is that no residual TBP was observed in the third hexane rinse (SI Table 2), providing an easy method for cleaning. The column was regenerated through thermal heating to 60 °C in order to remove all residual hexane (∼5 min) before returning to room temperature. In order to make the unit semiautonomous, the control of the check valves was automated (Figure 1), as

DOI: 10.1021/acssensors.9b01624
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detailed in the methods. After each collection step, ∼2 mL total of hexane is required for complete removal of contaminants from the preconcentrator (SI Table 2), and can be accounted for in a future, automated prototype.

Microreactor Design and Operation. A microfluidic reactor with immobilized enzymes (AChE, COX, and HRP) that convert ACh to a fluorescent byproduct was developed for semicontinuous operation; i.e. the enzyme and the fluidic reactor could be reused as long as (1) ACh and Amplex Red could be continuously delivered and (2) the device was not exposed to OPs or other AChE inhibitors, thus inactivating the enzyme. Therefore, a serpentine channel with width 200 μm and depth 400 μm was fabricated and biofunctionalized as detailed in the methods, and combined with a droplet generator to create a heterogeneous mixture of oil containing analyte and aqueous solution containing reaction media.

The amount of enzyme in the microfluidic bioreactor is critical to its sensitivity, responsiveness, and efficacy. For example, immobilization of excessive amounts of enzyme in the microreactor, while creating a rapid response, will also lead to generation of excessive reporter molecules, both saturating the detector as well as diluting the effect of OP inhibition; this results in a suboptimal limit of detection. To optimize the amount of enzyme immobilized, the flow rate, the mass flow, and the dwell time of the solution were tested; as expected, an asymptotic response was observed with respect to protein concentration lost from solution and flow rate (SI Figure 1). The final conditions chosen for immobilization were 30 μL/min for 2 h of enzyme cocktail (see Methods). A 1× PBS solution containing 5 μL/min of either 100 μM ACh and 100 μM amplex red, or 100 μM ACh and 1 mM of amplex red (10×) was then injected into the bioreactor, resulting in a fluorescence signal ∼75% of the saturated intensity of the detector. Since the reagents are only in the aqueous phase, we observe a patterned eluent in the microfluidic tubing (Figure 2C). The fluorescent eluent was measured (λ_{ex} = 550 nm and λ_{em} = 600 nm) in a cuvette and correlated back to TBP concentration. As low as 50 pmol of TBP were sufficient to cause reaction quenching (Figure 3A) demonstrating the high sensitivity (∼3000× improvement) of the assay compared to previous methods demonstrating detection of 150 nmol of TCP.[11] Accounting for the volume of air that would be sampled by our Air2Liquid device, 50 pmol would correspond to 2 ppb air concentration (mol/mol), assuming 100% collection and solubilization. Assuming a more likely 30% collection efficiency and 80% solubilization efficiency in the first milliliter (SI Table 2), this still corresponds to ∼10 ppb, demonstrating the sensitivity of the approach. As an added advantage, the current method can be applied across all AChE inhibitors. Due to the key step being tight inhibition of AChE, we envision this bioreactor being deployed as a replaceable cartridge, where an exposure event will trigger substitution of the cartridge to a new bioreactor with uninhibited AChE.

Integration of Miniaturized Optical Detector. The fluorescence intensity needs to be detected directly after the microfluidic reactor to enable pseudo real-time detection using a wearable, or at least transportable fluorescent detector. SI Figure 2 shows the scheme of the fluorescence detector and its operation, with the design of the detector mechanism based on that of a fluorescence microscope and previous photodetectors for biological systems.[20,21] The key parts are the excitation light source, barrier filter, polarizing filter, and photodetector that were down-selected from an original list (SI Table 2). A Green LED (550 nm) was selected and used as the excitation light source and combined with a 610 nm filter and a photodetector with high sensitivity at 620 nm (see Methods). Emission of the fluorescence substrate is known to be at 600 nm. However, the fluorescent signal could not be discriminated from the background light from the LED, even with the polarization filter, due to light scattering. Adding a 610 nm filter between the cuvette and the detector resulted in a clearer, more robust fluorescence response. The final device (see Methods) then had the fluorescence intensity recorded as current using data logging software. The efficacy of the detector was then demonstrated using a modified reaction mixture, containing 1 mM of Amplex Red in the reaction buffer (10×), SI Figure 2C. The microfluidic reactor with integrated detector was then tested using a flow-rate of 5 μL/min for both 1 mL of hexane impregnated with TBP, as well as the modified reaction mixture. As expected, a substantial change in fluorescence was observed upon exposure of the bioreactor to TBP (Time 50 s to Time 300 s).

Demonstration of Device from Vapor Collection to Detection. To verify that each component of the Air2Liquid device worked in conjunction with the others, we performed one final integrated test. Air was sampled from the head space of a 50 mL beaker filled with 10 mL TBP onto the μ-PC for 10 min; we estimate that ∼10 μg was collected. One mL of hexane was then washed through the μ-PC with an estimated 8.6 μg of TBP resolubilized (SI Table 2). The TBP impregnated hexane was then transferred to the microreactor. Both the hexane and reaction mixture were injected at 5 μL/min into the droplet generator/bioreactor for analysis.

The fluorescence changed as shown in Figure 3B at 400 s post TBP injection, produced nA changes in current due to reduced fluoresence. To eliminate the noise, a 1 Hz low band-pass filter was applied (Figure 3B, red line). We back-calculated the total number of pmol that results in an observable change in fluorescence to be no more than 600 pmol. When correcting for losses throughout the process (∼80% of the analyte being resolubilized in the first rinse and ∼30% capture efficiency), this corresponds to an estimated LOD of ∼100 ppb from sampled air. Therefore, this final experiment demonstrated the efficacy of a joined Air2Liquid device that could be integrated and automated for future deployment.

CONCLUSIONS

The need for cost-effective, on-site classification and quantitation of trace VOCs and sVOCs is critical. Our development of a novel Air2Liquid platform and corresponding proof-of-concept study provides one path toward meeting this need. In short, we demonstrated (1) an air sampling device that collects hydrophobic sVOCs (i.e., OPs), (2) transfer of the sVOCs into a compatible organic solvent, and (3) integration into an enzymatic assay. In future iterations, we believe that the first two stages of the device can be further optimized to collect and concentrate any hydrophobic sVOC or aerosolized compound, while the assay can then be modified for detection of the compound of interest.

Substantial integration and automation work remains to create a field-deployable prototype that can be used outside of the laboratory. Future iterations will also explore reference channels for long-term enzyme degradation, as well as electrochemical reactors to identify oxidizers present, such as

DOI: 10.1021/acssensors.9b01624
ACS Sens. 2020, 5, 13−18

acetone peroxide, that may react with Amplex Red and lead to false positives.

Regardless, this initial proof-of-concept study where we detect TBP using our novel Air2Liquid method demonstrates the efficacy of the approach. We believe this method enables a new class of diagnostic systems that combine a μ-PC with microfluidic assays for selective and sensitive detection of gaseous analytes of interest.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.9b01624.

Additional information about collection and fabrication (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Mitchell.Rubenstein@us.af.mil.

ORCID

Michael C. Brothers: 0000-0002-8239-2399

Steve S. Kim: 0000-0002-9519-077X

Notes

The authors declare the following competing financial interest(s): P.L. and D.A. are cofounders of Defiant Technologies, a company specializing in environmental and CBRNE detection.

■ ACKNOWLEDGMENTS

We would like to thank Defense Health Programs for funding this project under contract #FA8650-14-D-65616-0003. We would like to thank Drs. Darrin Ott and Claude C. Grigsby for their support and encouragement, as well as Mr. Will Bell & Dr. R. Craig Murdoch who provided program and financial management. The views expressed in this article are those of the author and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the U.S. Government.

■ REFERENCES

(1) Schindler, B. K.; Koslitz, S.; Weiss, T.; Broding, H. C.; Brüning, T.; Bünger, J. Exposure of Aircraft Maintenance Technicians to Organophosphates from Hydraulic Fluids and Turbine Oils: A Pilot Study. *Int. J. Hyg. Environ. Health* **2014**, *217* (1), 34−37.

(2) Colovic, M. B.; Krstic, D. Z.; Lazarevic-Pasti, T. D.; Bondzic, A. M.; Vasic, V. M. Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. *CN* **2013**, *11*, 315.

(3) Storm, J. E.; Rozman, K. K.; Doull, J. Occupational Exposure Limits for 30 Organophosphate Pesticides Based on Inhibition of Red Blood Cell Acetylcholinesterase. *Toxicology* **2000**, *150* (1−3), 1−29.

(4) Spinelle, L.; Gerboles, M.; Kok, G.; Persijn, S.; Sauerwald, T. Performance Evaluation of Low-Cost BTEX Sensors and Devices within the EURAMET Key-VOCs Project. *Proceedings* **2017**, *1* (4), 425.

(5) Fernández-Maestre, R. Ion Mobility Spectrometry: History, Characteristics and Applications. *Rev. U.D.C.A Act. & Div. Cient.* **2012**, *15* (2), 467−479.

(6) Mujahid, A.; Dicker, F. L. Surface Acoustic Wave (SAW) for Chemical Sensing Applications of Recognition Layers. *Sensors* **2017**, *17* (12), 2716.

(7) Sferopoulos, R. *A Review of Chemical Warfare Agent (CWA) Detector Technologies and Commerical-Off-The-Shelf Items*; Human Protection and Performance Division; DSTO Defence Science and Technology Organisation: Victoria, Australia, 2008; pp 1−98.

(8) Harshman, S. W.; Rubenstein, M. H.; Qualley, A. V.; Fan, M.; Geier, B. A.; Pitsch, R. L.; Slusher, G. M.; Hughes, G. T.; Dershem, V. L.; Grigsby, C. C.; et al. Evaluation of Thermal Desorption Analysis on a Portable GC−MS System. *Int. J. Environ. Anal. Chem.* **2017**, *97* (3), 247−263.

(9) Ouyang, G.; Pawliszyn, J. SPME in Environmental Analysis. *Anal. Bioanal. Chem.* **2006**, *386* (4), 1059−1073.

(10) Wang, J.; Satake, T.; Suzuki, H. Microfluidic Device for Coulometric Detection of Organophosphate Pesticides. *Anal. Sci.* **2015**, *31* (7), 591−595.

(11) Yang, X.; Zitova, A.; Kirsch, J.; Fergus, J. W.; Overfelt, R. A.; Simonian, A. L. Portable and Remote Electrochemical Sensing System for Detection of Tricresyl Phosphate in Gas Phase. *Sens. Actuators, B* **2012**, *161*, 564−569.

(12) Xu, T.; Wang, J.; Wang, X.; Slawecki, R.; Rubio, F.; Li, J.; Li, Q. X. Comparison of Four Commercial Enzymatic Assay Kits for the Analysis of Organophosphate and Carbamate Insecticides in Vegetables. *Food Control* **2012**, *27* (1), 94−99.

(13) Erhard, M. H.; Kühlmann, R.; Szinicz, L.; Lösch, U. Detection of the Organophosphorus Nerve Agent Soman by an ELISA Using Monoclonal Antibodies. *Arch. Toxicol.* **1990**, *64* (7), 580−585.

(14) Mionetto, N.; Marty, J.-L.; Karube, I. Acetylcholinesterase in Organic Solvents for the Detection of Pesticides: Biosensor Application. *Biosens. Bioelectron.* **1994**, *9* (6), 463−470.

(15) Soares, C. M.; Teixeira, V. H.; Baptista, A. M. Protein Structure and Dynamics in Nonaqueous Solvents: Insights from Molecular Dynamics Simulation Studies. *Biophys. J.* **2003**, *84* (3), 1628−1641.

(16) Lee, J. N.; Park, C.; Whitesides, G. M. Solvent Compatibility of Poly(Dimethylsiloxane)-Based Microfluidic Devices. *Anal. Chem.* **2003**, *75* (23), 6544−6554.

(17) Meller, K.; Szumski, M.; Buszewski, B. Microfluidic Reactors with Immobilized Enzymes—Characterization, Dividing, Perspectives. *Sens. Actuators, B* **2017**, *244*, 84−106.

(18) Santillo, M. F.; Liu, Y. A Fluorescence Assay for Measuring Acetylcholinesterase Activity in Rat Blood and a Human Neuroblastoma Cell Line (SH-SY5Y). *J. Pharmacol. Toxicol. Methods* **2015**, *76*, 15−22.

(19) Baig, S. A.; Akhtera, A.; Ashfaq, M.; Asi, M. R. Determination of the Organophosphorus Pesticide in Vegetables by High-Performance Liquid Chromatography. *J. Agric. Environ. Sci.* **2009**, *6* (5), 513−519.

(20) Wu, J.; Liu, X.; Wang, L.; Dong, L.; Pu, Q. An Economical Fluorescence Detector for Lab-on-a-Chip Devices with a Light Emitting Photodiode and a Low-Cost Avalanche Photodiode. *Analyst* **2012**, *137* (2), 519−525.

(21) Yotter, R. A.; Wilson, D. M. A Review of Photodetectors for Sensing Light-Emitting Reporters in Biological Systems. *IEEE Sens. J.* **2003**, *3* (3), 288−303.

DOI: 10.1021/acssensors.9b01624
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