Vapor Phase Detection of a Narcotic Using Surface Acoustic Wave Immunoassay Sensors

Desmond D. Stubbs, Sang-Hun Lee, and William D. Hunt

***Abstract*—Currently, the narcotic sniffing dog remains the most accurate, reliable, and widely used sensing technology in the war on drugs. However, recent studies done at the Institute for Biological Detection Systems at Auburn University, Auburn, AL, have shown that in the presence of extraneous odors (nontarget odors), these animals show a higher propensity for so-called false alarms. For this reason, there have been an increasing demand for a portable, highly specific vapor-sensing device capable of distinguishing a target vapor signature in a complex odor. In this paper, we present the results of a series of experiments demonstrating real-time vapor phase detection of cocaine molecules. A distinctive response or signature was observed under laboratory conditions, where the cocaine vapors were presented using an INEL vapor generator and under "field" conditions facilitated by the Georgia Bureau of Investigation Crime Lab. For these experiments, the sensor component was an ST-X quartz resonator with a center frequency of approximately 250-MHz. anti-benzoylecgonine (anti-BZE) antibodies are attached to the electrodes on the device surface via a protein-A cross linker. We observed a large transient frequency shift accompanied by baseline shift with the anti-BZE coated sensor. After repeated experiments and the use of numerous controls, we believe that we have achieved real-time molecular recognition of cocaine molecules.**

***Index Terms*—Cocaine detection, immunoassay, monoclonal antibody, surface acoustic wave (SAW) sensor.**

I. Introduction

THERE have been numerous laboratory studies designed to determine the mechanism by which odor-sensing dogs identify a specific chemical target [1]–[3]. These studies indicate that under normal laboratory conditions, there is a high degree of accuracy among the dogs with detection limits in the sub-parts-per-billion range. Unfortunately, under field conditions, the problem become increasingly more complex due to the presence of extraneous odors created by the purveyors of these illicit materials in an attempt to thwart detection. With the introduction of these extraneous odors, the dogs' ability to detect a target odor was considerably hampered due to its inability to differentiate one odor over another in a complex chemical vapor. The highly specific and complex nature of olfactory sensing systems has lead to the development of vapor phase chemical detection systems that go by the name of "electronic nose." These systems usually consist of some chemical- or biological-sensing component attached to a transducer. Throughout the current literature, surface acoustic wave (SAW) devices have been increasingly incorporated into these sensing systems. SAW-based devices has been used for various applications ranging from communications to its more recent function as a biosensor. In a series of papers, Guilbault and fellow researchers [4]–[6] reported the use of films of biomolecules, such as enzymes and antibodies on quartz–crystal microbalance (QCM) devices, for vapor phase detection of formaldehyde and organophosphorous pesticides such as parathion. Subsequent studies by others were unable to confirm the specificity reported by Guilbault. Rajakovic *et al.* [7] found that sensors coated with anti-parathion antibodies showed sensitivities to malathion, parathion, and disulfoton that were not markedly different from the response of sensors coated with proteins (valproic acid antiserum, bovine serum albumin, and human IgG) containing no specific binding sites for these analytes. In addition, one could also conclude that the anti-parathion QCM immunoassay sensor of Ngeh-Ngwainbi, *et al.* [5] does not indicate antigen-antibody binding activity. The two main features of a functional vapor-sensing device are sensitivity and specificity. The sensitivity is prescribed by the sensor modality and design and the degree of specificity is dependent on the detailed nature of the chemically sensitive film on the surface of the device. These devices and systems are currently being used in biotechnology, food industry, medicine, environment, and, most recently, law enforcement applications. Interdiction efforts continue in the search for technologies which can provide an inexpensive alternative to dogs as detectors of narcotics and explosives. One of the principle motivations for the development of electronic noses for these applications is the expense associated with of the handlers and training and care for the dogs. Further, it is still unclear as to what, chemically, the dogs are actually detecting, and this may vary from dog to dog. Consequently, all dogs do not respond to the same cocaine sample. Although the dogs have proved to be a highly useful tool in detecting illicit materials, as a tool for analytical chemistry, they leave one wanting. One would never accept data from an instrument without having a solid idea of the physical mechanism behind a detection event.

Herein, we used anti-benzoylecgonine (anti-BZE) as our sensing layer for the detection of cocaine molecules contained in a vapor stream. Cocaine and benzoylecgonine are structural analogs, with the latter being the predominant metabolite of the former.They differ only by a methyl group that forms a methyl

Manuscript received April 9, 2003; revised December 2, 2003. The associate editor coordinating the review of this paper and approving it for publication was Prof. Michael Pishko.

D. D. Stubbs is with the School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332 USA (e-mail: desmond.stubbs@chemistry.gatech.edu).

S.-H. Lee and W. D. Hunt are with the School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA 30332 USA (e-mail: gt6361b@prism.gatech.edu; Bill.Hunt@ee.gatech.edu).

Digital Object Identifier 10.1109/JSEN.2005.844541

ester with C′12 in cocaine that is cleaved when hydrolyzed to form the acid derivative in benzoylecgonine. Researchers have analyzed the headspace vapor of various cocaine samples and have found it to contain a concentration dependent upon the vapor pressure, temperature, and nature of the sample (Lawrence, 1985) [8]. However, the complete vapor composition has not been revealed and cannot consistently be agreed upon. In our work, we report the detection of cocaine vapors both in our lab using an Idaho National Engineering Laboratory (INEL) vapor generator loaded with pure cocaine sample and in the Georgia Bureau of Investigation (GBI) using seized "crack" cocaine samples.

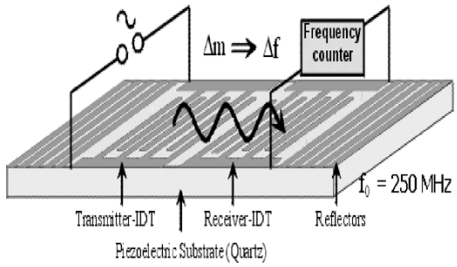
For our sensor modality, we have selected SAW quartz resonator devices that are designed for vapor phase detection. The mode of acoustic propagation is determined by electrode geometry and by the particular cut of crystal used. Both the acoustic velocity and attenuation are affected by the selective medium.

II. Methodology

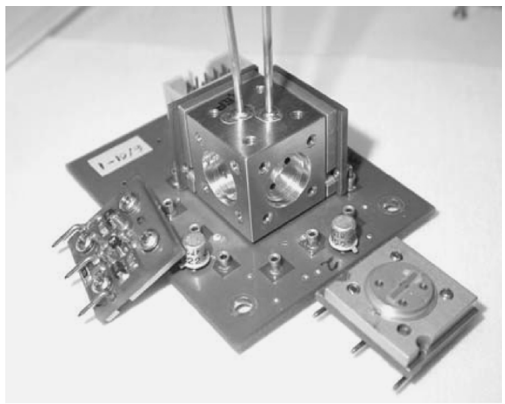
Our approach is to construct a vapor phase biosensor by immobilizing a monolayer of antibodies onto the surface of a SAW device as is shown schematically in Fig. 1(a). The device is then connected into an oscillator circuit, as shown in Fig. 1(b), and frequency changes can then be precisely measured. When the antigen binds to the antibody, the acoustic velocity is altered and the oscillator frequency shifts to different value (Fig. 2). As a control, we used both anti-FITC coated and hydrogel-coated devices and compared the responses, the reason being that both anti-BZE and anti-FITC monoclonal antibodies are specific for small molecules which have molecular weights in the range of 300 Daltons. In addition, the two molecules, cocaine and FITC, may possess a hydrophobic epitope. Further, to overcome the problem of dehydration of the biomolecular film, we have developed a method for applying a thin hydrogel layer over the immobilized antibodies. For our devices, we have obtained sensitivities of approximately 20 Hz/pg with a detection limit on the order of a few picograms. A picture of our SAW resonator array is shown in Fig. 1(b).

A. Antibody Immobilization

The antibody immobilization technique first requires polarizing the electrode surface. This was accomplished by immersing the SAW chip in 5 ml of 1.2-M HCl for 5 min then washing with deionized water, followed by 5 ml of 1.2-M NaOH for 5 min (according to the method developed by Davis and Leary) [9]. The SAW chip was washed again with deionized water before it was immersed in 3 ml of 1.2-M HCl for 2 min. The chip was washed twice with deionized water followed by buffer solution and was allowed to air dry. Protein A (0.2 mg) was dissolved in 100 μl of buffer solution at physiological pH. Then, 10 μl of antibody (0.023 mg/ml) was mixed with the buffered protein-A solution and the mixture was placed in a 4 °C refrigerator for 2 h. Three microliters of the cross-linker-antibody solution was used to coat the device. The chip was allowed to air dry for 1 h and the remaining solution was spun off for 30 s at 5000 rpm. A thin layer of hydrogel (3 μl) was then applied by spinning the chip on a silicon wafer for 50 s at 5000 rpm. We obtained both the mouse monoclonal anti-FITC antibodies and Protein A-soluble (extracellular) from Sigma Chemical Company, St. Louis, MO. Uranine was obtained from Fluka Chemika. Mouse anti-benzoylecgonine was purchased from Research Diagnostics, Inc., Flanders, NJ. This lot of antibody was found to cross react to benzoylecgonine and cocaine. Benzoylecgonine is the major metabolite of cocaine found in the blood stream.



(a)



(b)

Fig. 1.(a) Simplified diagram of a typical two-port SAW resonator. (b) Packaged resonators in circuit oscillator and flow system. Sensor head holds and facilitates the simultaneous sampling of four sensors.

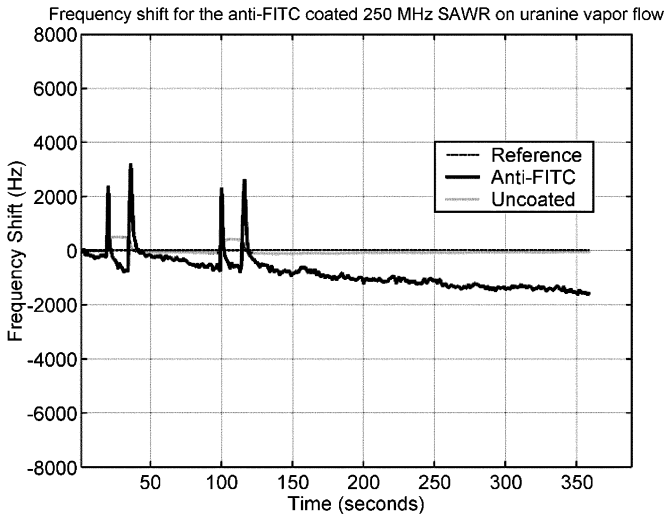


Fig. 2. Anti-FITC-coated SAW response to uranine (soluble FITC salt) vapor flow. For analyte presentation, N_2 (g) was bubbled through a 1-nM solution of the uranine at a flow rate of 0.5 slpm (standard liters per minute). N_2 (g) flow was continuous during the experiment as analyte vapor samples were pulsed into the stream for two different 15-s periods (20–35 s and 100–115 s).

B. SAW Resonator Device Description

The experiments described herein were conducted using two port SAW resonators fabricated in the laboratories of the Microelectronic Acoustics Group at the Georgia Institute of Technology (Georgia Tech), Atlanta. These devices have a center frequency of nominally 250-MHz two-port resonators with Al electrode metallization on ST-X quartz substrates. ST-X quartz is a particular cut of quartz for which interdigital transducer (IDT) structures generate more or less a Rayleigh wave in the substrate material. More importantly, for sensor applications, the ST-X cut of quartz is known to provide very high-temperature stability near room temperature [10], which minimizes the need for precise temperature control. In Fig. 1(a), we present a diagram of a typical two-port SAW resonator. The gratings on either side of the IDTs form a resonant acoustic cavity. One IDT acts as an input transducer which converts the driving RF signal into an acoustic standing wave within the cavity. The output IDT converts the acoustic wave back into an RF signal which is fed back into the attendant oscillator circuitry. Two important parameters in the design of the SAW resonator are $\mathrm{d_g}$ and $\mathrm{d_m}$, the distance between grating and IDT and the distance between two IDTs, respectively. To maintain the standing waves in the cavity region, $\mathrm{d_g}$ and $\mathrm{d_m}$ should be, respectively

$$d_g = \frac{\lambda}{8} + \frac{n\lambda}{2}, \quad (n = 0, 1, 2, 3 \ldots) \tag{1}$$

$$d_m = \frac{\lambda}{4} + \frac{n\lambda}{2}, \quad (n = 0, 1, 2, 3 \ldots). \tag{2}$$

Since our protocol for antibody immobilization is expected to ensure that the antibodies attach to the metal electrodes (fingers) but not to the quartz surface, we designed our resonators to minimize the amount of bare quartz in the cavity region. This would also help us to achieve a uniform distribution of biomolecules in the SAW resonators' most sensitive region. Selecting the minimum possible value for $\mathrm{d_m}$ can aid in the achievement of this goal. For our SAW resonators, there are 500 fingers in each of the gratings and these ideally have widths of $\lambda/4$ where λ is the acoustic wavelength at the center frequency of the resonator. Generally, the IDT finger width is same as that of gratings. In our design, however, split-finger IDTs are used so as to reduce SAW reflections from the IDTs within the resonant cavity. Hence, the size of the IDT finger widths and the spacing between IDT fingers is 1.5 μm. This is the minimum feature size of our devices and it is one that, with care, can be achieved in our facilities. Each IDT in this design has 200 metal fingers. The main features of the SAW resonators used in this experiment are summarized in Table I.

Briefly, our fabrication procedures are as follows. After proper surface cleaning and treatment, Shipley S1805 positive photoresist was spun at 3200 rpm for 20 s and the device pattern was exposed to UV light for 10 s at the intensity of 8 mW/cm^2. Developing was done with the Microposit MF351 5:1 solution for 15–20 s. Two different metal layers were deposited using a CVC electron beam evaporator. An adhesion layer of 300 Å of Cr was deposited as a first layer followed by 1200 Å of Al. For the devices in these experiments where gold electrodes are used, an additional 300 Å of Au was deposited on top of the Al layer. After a liftoff process using acetone, the wafer was diced with a 3-mil-thick diamond-coated high-speed rotating blade. Then, each die was coated with the proper biofilm and mounted on a TO-8 header, which has a 12.7-mm (0.5″) diameter.

TABLE I
SAW RESONATOR DEVICE PARAMETERS

Wafer material	ST-X Quartz
Acoustic aperture (W)	850 μm
IDT-grating gap(dg)	7.8 μm
IDT-IDT gap (dm)	3.1 μm
IDT pitch (λ/2)	6.2 μm
IDT finger width	1.5 μm
Grating pitch (λ/2)	6.2 μm
Grating finger width (λ/4)	3.1
Metal film thickness	1500 Å
Diced chip size	8100x1980 μm

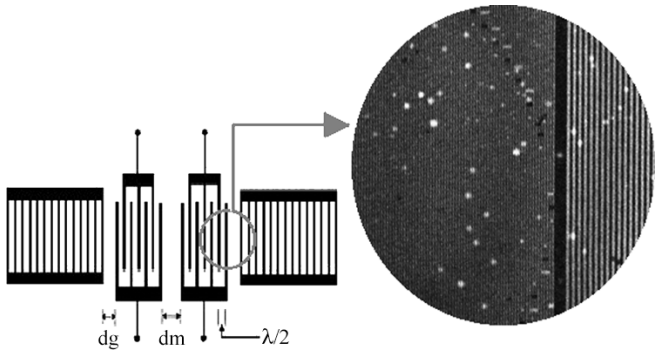


Fig. 3. CLSM image of uranine attached to the SAW electrodes. The image represents the IDT and gratings portion of electrode geometry. Fluorescent molecules are observed on the metal electrode (green due to reflection) and not the quartz (black due to light absorption), as dictated by the immobilization procedure.

III. RESULTS

As indicated previously, in order to verify vapor phase analyte binding events, we developed a fluorescent antibody/analyte assay. This was to provide a method, independent of the SAW device response, to detect the occurrence of a molecular binding event. SAW devices with and without antibody films were tested. After brief exposure to the analyte vapor, the TO-8 packaged device was pulled from the system, washed with buffer to remove unbound analyte and then viewed using a Zeiss LSM510 confocal fluorescent microscope (CLSM) located in the Institute for Bioengineering and Biosciences, Georgia Tech. If fluorescence was observed in the CLSM image, this is evidence of bound fluorescent analyte (Fig. 3).

A. Device "Field" Test

Under the supervision of the GBI's chemical analysis scientist, we investigated the headspace of a seized sample being processed using the SAW Pro as the mode of presenting the vapor to the sensor head. The sample was believed to be cocaine freebase, commonly called "crack." Vapor signature analysis revealed the characteristic device response commonly encountered under laboratory conditions. As a control, we used nonspecific anti-FITC antibodies and observed no such response. In addition, the response was only observed when held above

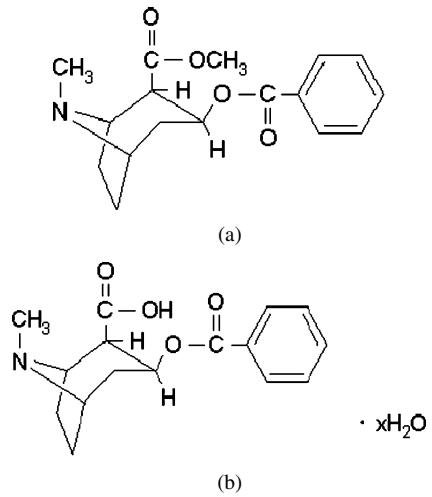


Fig. 4. (a) Structure of a cocaine molecule. (b) Structure of a benzoylecgonine molecule.

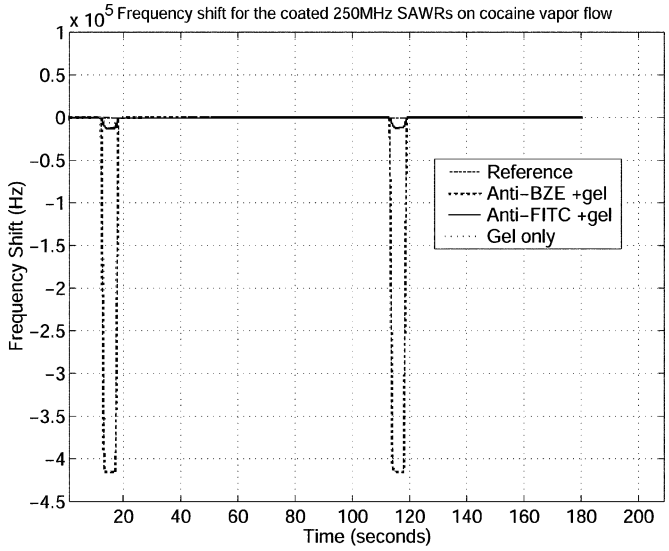


Fig. 5. Antibody-coated and gel-coated device response to INEL cocaine vapor generator. Using a cocaine vapor generator specifically designed to release a precise amount of cocaine at a calibrated temperature and flow rate, two 5-s pulses of a 1-ng cocaine sample was presented over a 100-s interval to the sensor head. The pulse was injected into a constant flow of 180 ccm to minimize the impact associated with a sudden pressure differential. The oscillating circuit housed an anti-FITC/gel coated device, a gel-only coated device, an anti-BZE/gel coated device and a reference device. As observed, the response of the anti-BZE/gel coated device was far more dramatic (∼40-fold differential) than the response of the other devices and that there was little or no difference between the gel-only coated device and the anti-FITC/gel coated device.

the sample headspace indicative of the very low vapor pressure cocaine.

B. Vapor Presentation

The INEL vapor generator is calibrated to release a specific amount of cocaine when a particular temperature, flow rate and pulse time is entered (data available at Houston's Research Laboratories). Cocaine pellets housed within a separate compartment are heated at a constant temperature that corresponds to a known amount of cocaine molecules (Fig. 4) that can be manually or automatically pulsed into the clean air flow. In this

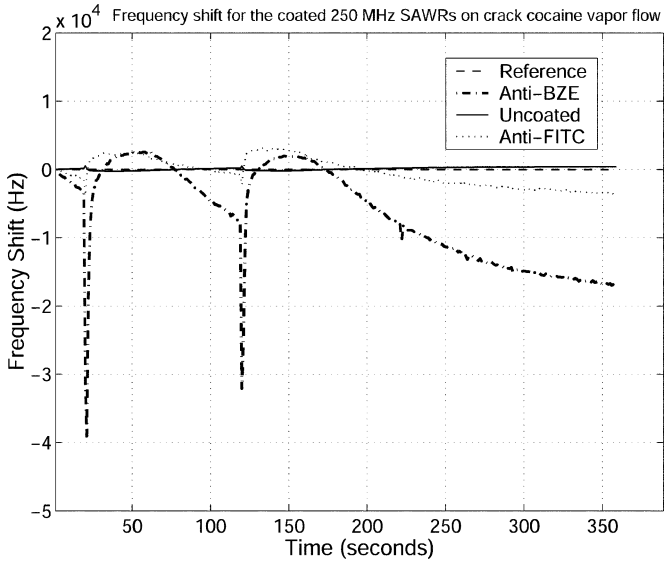


Fig. 6. Response of anti-BZE/gel coated device during cocaine presentation. Here, the anti-BZE/gel coated device recovers to shifted baseline (∼20 kHz) due to the mass loading effect. In this experiment, an anti-FITC/gel coated resonator and an uncoated device were used as negative controls and displayed a relatively minimal response to the presentation of cocaine vapors. A reference device (uncoated) which was subjected to the same temperature and pressure environment as the other three resonators was used to subtract out the effect of these fluctuation to the overall response.

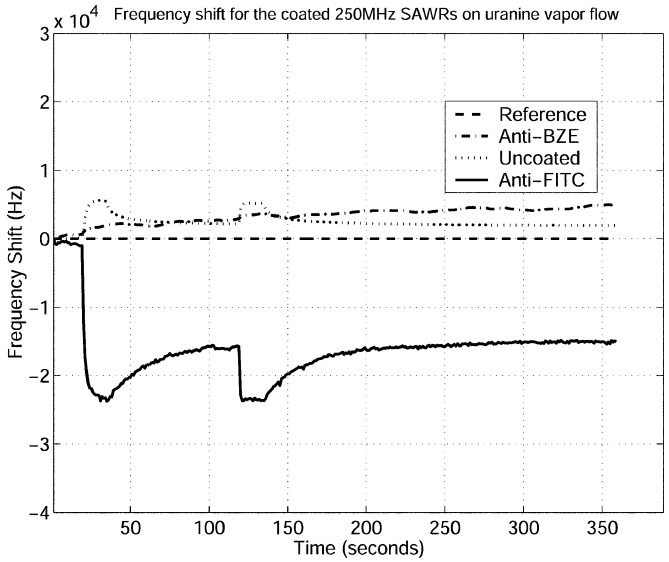


Fig. 7. Effect of uranine vapor flow on anti-BZE, anti-FITC, and an uncoated device arranged in a SAW sensing array. Again, for analyte presentation, N_2 (g) was bubbled through a 1-nM solution of the uranine at a flow rate of 0.5 slpm (standard liters per minute). N_2 (g) flow was continuous during the experiment as analyte vapor samples were pulsed into the stream for two different 15-s periods (25–40 s and 125–140 s).

case, the automated vapor generator was programmed to deliver ∼1 ng of cocaine vapor to a flow cell containing four surface acoustic wave resonators in oscillating circuits. The experiments begin with a flow of clean air (180 ccm) through the flow cell while monitoring the resonant frequency of the SAW devices in their respective oscillator circuits. This allowed us to account for any response to temperature and pressure changes during presentation of the analyte gas. A 5-s pulse of cocaine is injected into air stream and presented to the flow cell.

The important variables are flow rate (180 $\pm$ 0.3 ccm), temperature, and pressure, these values are held constant regardless of the pulse. In other words, the addition of cocaine molecules to the air stream during the pulses does not modulate the flow rate or cause a significant variance in temperature and pressure values. Therefore, any frequency shift after the initial onset of airflow cannot be attributed to changes in aerial quality, but is solely due to the perturbation of the device surface by the cocaine molecules mixed in the airflow, whether the shift is from the specific binding events or not. The results (shown in Figs. 5–7) were compared to the data published in previous work done by Stubbs *et al.*, [11], [12], where anti-uranine antibodies were immobilized on SAW devices for the detection uranine molecules in an air stream (Fig. 3).

IV. Discussion

Vapor signature analysis revealed the characteristic device response commonly encountered under laboratory conditions. As a control, we used nonspecific anti-FITC antibodies and observed no such response. In addition, the response was only observed when held above the sample headspace indicative of the very low vapor pressure cocaine. We believe the characteristic response is determined not only by device design, but also by molecular; that is, we believe the initial transient observed is due to mass loading and some changes in the spring constant of the biolayer. This explanation, however, does not explain the differences in the magnitude of response; we believe that this difference may be a function of the technique used to deliver the vapors to the sensor head. For instance, the large response was observed when we had constant air flow and cocaine was pulsed into the air stream. The constant airflow (180 ccm) may have a drying effect on the gel coating and may exaggerate the mass-loading effect, which translate into a larger-than-normal transient. Evidence for this is contained in the anti-FITC response that showed a 20-fold decrease in magnitude. The gel-coated device showed a similar response to the anti-FITC/gel coated device.

Acknowledgment

The authors would like to thank S. Woodard of the Georgia Tech's Institute for Bioengineering and Biosciences for training them on the Zeiss LSM 510. They would also like to thank D. Hoglund of the U.S. Customs Service for his support of this program, as well as T. Mills, III, M. D. Burns, and T. Wu at GBI Crime Laboratory for their support.

References

[1] P. L. Woggonner, M. Jones, M. Williams, J. M. Johnston, C. Edge, and J. A. Petrousky, "Effects of extraneous odors on canine detection of illicit drugs," in *Proc. 2nd Annu. Conf. Enforcement and Security Technology*, Bellingham, WA, 1998, pp. 1–8.

[2] J. M. Johnston, Canine Detection Capabilities: Operational Implications of Recent R & D Findings, Inst. Biol. Dectection Syst., Auburn Univ., Auburn, AL, 1999.

[3] M. Williams, J. M. Johnston, L. P. Waggoner, M. Cicoria, S. F. Hallowell, and J. A. Petrousky, "Canine substance detection: operational capabilities," presented at the The Office of National Drug Control Policy Technology Symp., 1999, pp. 1–11.

[4] G. G. Guilbault, "Determination of formaldehyde with an enzyme-coated piezoelectric crystal detector," *Anal. Chem.*, vol. 55, pp. 1682–1684, 1983.

[5] J. Ngeh-Ngwainbi, P. H. Foley, S. S. Kuan, and G. G. Guilbault, "Parathion antibodies on piezoelectric crystals," *J. Amer. Chem. Soc.*, vol. 108, pp. 5444–5447, 1986.

[6] G. G. Guilbault and J. H. Luong, "Gas phase biosensors," *J. Biotechnol.*, vol. 9, pp. 1–10, 1988.

[7] L. Rajakovic, V. Ghaemmaghami, and M. Thompson, *Anal. Chim. Acta*, vol. 217, p. 111, 1989.

[8] A. H. Lawrence and L. Elias, "Determination of ecgonidine methyl ester vapor pressure using a dynamic gas blending system and gas chromatography analysis," *Anal. Chem.*, vol. 69, pp. 4283–4285, 1997.

[9] K. A. Davis and T. R. Leary, *Anal. Chem.*, vol. 61, pp. 1227–1230, 1989.

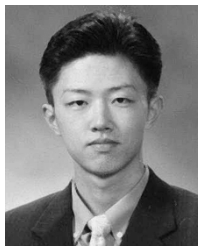
[10] C. S. Lam and D. E. Holt, "A comparison of temperature performance of SAW filters made on ST- and LST-cut quartz," in *Proc. IEEE Int. Ultrasonics Symp.*, Chicago, IL, Oct. 1988, pp. 273–277.

[11] D. D. Stubbs, W. D. Hunt, S. H. Lee, and D. F. Doyle, "Gas phase activity of anti-FITC antibodies immobilized on a surface acoustic wave resonator," *Biosens. Bioelectron.*, vol. 17, no. 6–7, pp. 471–477, June 26, 2002.

[12] ——, "Vapor phase SAW immunoassay sensors," in *Proc. IEEE Int. Ultrasonics Symp.*, vol. 1, Oct. 2001, pp. 357–360.

Desmond D. Stubbs was born in Nassau, Bahamas, 1972. He received the B.S. degree in chemistry from Morris Brown College, Atlanta, GA, in 1997 and the M.S. degree in biochemistry from the Georgia Institute of Technology, Atlanta. He is currently pursuing the Ph.D. degree at the School of Chemistry and Biochemistry, Georgia Institute of Technology. His doctoral thesis involves engineering proteins for biosensor applications.

From 1999 to 2001, he was a demonstrations Teacher in the School of Chemistry, Georgia Institute of Technology.



Sang-Hun Lee was born in Seoul, Korea, on October 7, 1970. He received the B.Eng. degree in electronics engineering from Korea University, Seoul, and the M.S. degree in electrical and computer engineering from the Georgia Institute of Technology, Atlanta, in 1998 and 2000, respectively. He is currently pursuing the Ph.D. degree in electrical engineering at the Georgia Institute of Technology as a Graduate Research Assistant.

His main research interest is in the design and fabrication of various acoustic wave devices for high-sensitivity chemical and biosensors.

William D. Hunt received the B.S. degree from the University of Alabama, Tuscaloosa, in 1976, the M.S. degree from the Massachusetts Institute of Technology (MIT), Cambridge, in 1980, and the Ph.D. degree from the University of Illinois, Urbana-Champaign, in 1987, all in electrical engineering.

Prior to his entrance into MIT's graduate program, he was an Engineer with Harris Corporation. Following completion of the M.S. degree, he joined the engineering staff at Bolt Beranek and Newman Corporation. He joined the electrical engineering faculty at the Georgia Institute of Technology, Atlanta, following completion of the Ph.D. degree.

Dr. Hunt received the NSF Presidential Young Investigator Award in 1989, the DuPont Young Faculty Award in 1988, and the University of Alabama Distinguished Engineering Fellowship in 1994. He was a Rhodes Scholar Finalist in 1975. His area of expertise is in the area of microelectronic acoustic devices for wireless applications, as well as chemical and biological sensors based on this technology. He has published over 70 papers in refereed journals and conference proceedings. He holds four U.S. patents and five provisional patents.