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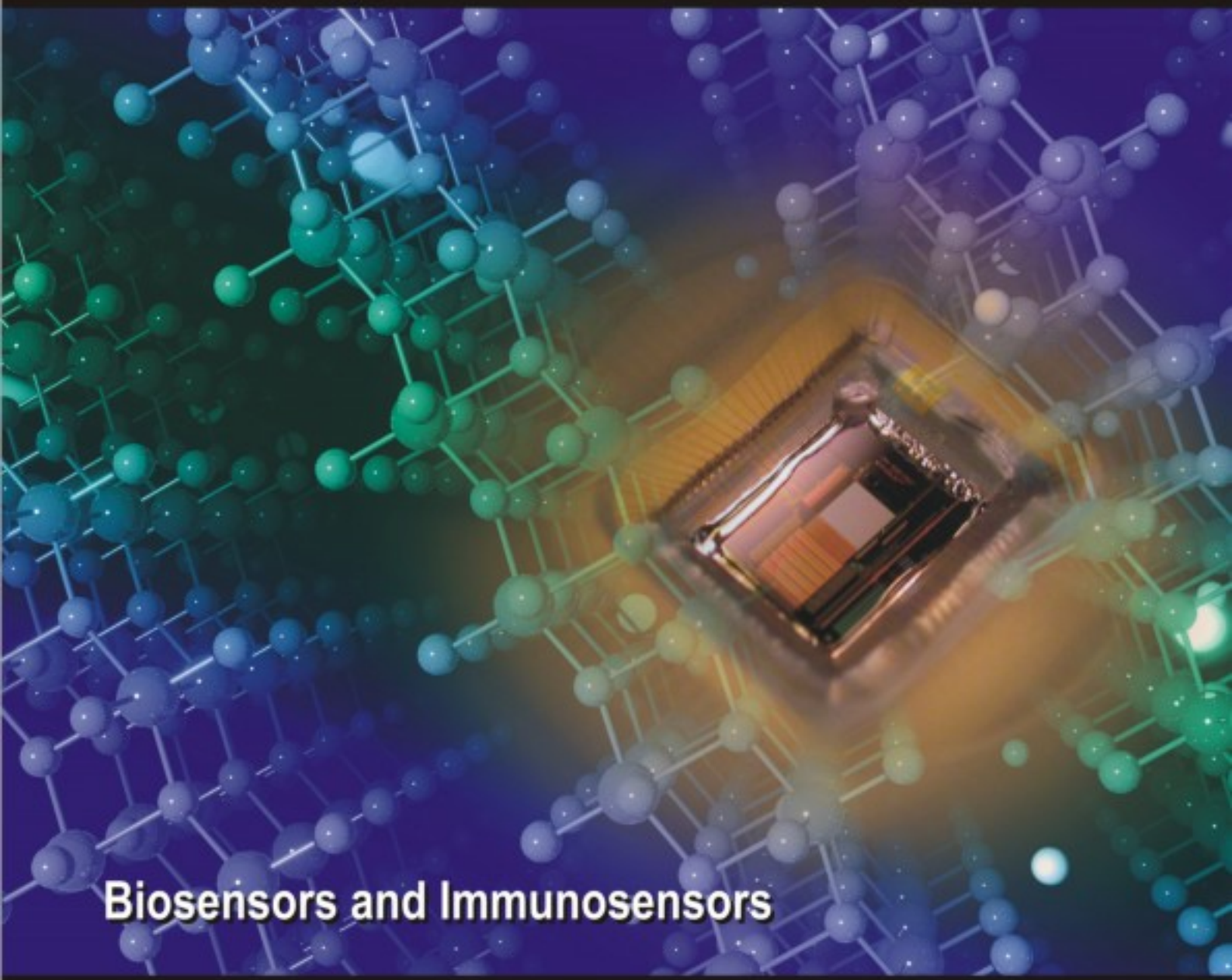

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Embedded Piezoresistive Microcantilever Sensors Functionalized for the Detection of Methyl Salicylate

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Abstract: Sensors designed to detect the presence of methyl salicylate (MeS) have been tested. These sensors use a sensor platform based on the embedded piezoresistive microcantilever (EPM) design. Sensing materials tested in this study included the polymer poly (ethylene vinyl acetate), or PEVA as well as a composite sensing material consisting of the enzyme SA-binding protein 2, or SABP-2. The SABP-2 was immobilized within a biocompatible Hypol gel matrix. The PEVA-based sensors exhibited slower but reversible responses to MeS vapors, recovering fully to their initial state after the analyte was removed. SABP-2 sensors exhibited faster overall response to the introduction of MeS, responding nearly instantly. These sensors, however, do not recover after exposures have ended. Sensors using the SABP-2 sensing materials act instead as integrating sensors, measuring irreversibly the total MeS dose obtained. *Copyright © 2013 IFSA.*

Keywords: Microcantilever, Methyl salicylate, Piezoresistive, SABP-2, Biosensor.

1. Introduction

Microcantilever-based chemical or biological sensors exist in a number of different configurations [1, 2]. In most of these configurations, the basic principle is the same: the presence of the analyte causes a tiny semiconductor microcantilever to bend slightly. This bending is usually detected by direct optical measurement of the cantilever position, or through a resistance change in the cantilever itself as it bends. In different applications, the microcantilever may be essentially static, while in other applications the microcantilever is oscillating at or near one of its resonant frequencies. In the specific case of an oscillating cantilever, a change in the frequency or amplitude of oscillation may also indicate the presence of the analyte. In order for a specific analyte to be detected, the microcantilever must be functionalized with a sensing material. This material may be chemical or biological in nature, or both, depending on the specific sensing application. The design of the sensing material in most cases determines both the sensitivity and the specificity of the sensor, as the basic microcantilever platforms remain similar throughout most sensing applications. Applications of microcantilever sensors have been reported where the cantilevers are coated on one of their surfaces with a sensing material, or are in direct contact or embedded within with the sensing material [3, 4].

In the embedded piezoresistive microcantilever (EPM) platform [5, 6], microcantilever bending is measured through direct resistance measurement of a piezoresistive microcantilever. This platform greatly simplifies data collection electronics, as only a single

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resistance measurement, typically with 24-bit accuracy, is needed. This relative ease of interfacing the sensors to data collection devices makes them easy to use in applications where small size, rigidity, and low cost are important. These sensors may be easily interfaced to computers or handheld devices, or integrated as part of a wireless mesh network of sensors [7, 8]. In general, these EPM sensors are functionalized by embedding or partially embedding the tiny piezoresistive microcantilever into a custom designed sensing material during sensor fabrication. In some cases, the microcantilever may simply be pre-loaded atop a small bead of sensing material during assembly. The sensing material is typically synthesized so as to respond volumetrically to the presence of the target chemical or biological analyte. Sensing materials used in EPM applications may include common organic polymers, composite polymer/biomolecule materials, or polymers functionalized with other active particles or chemicals. In the case of simple polymers, liquid or vapor analytes are matched to the sensor by matching the solubility parameter of the polymer to the analyte. For the detection of a broad array of analytes, several sensors may be used in an array, with each potential analyte producing a unique signature pattern from the sensor array. In the EPM sensor, exposure to the desired analyte causes the sensing material volume to change, inducing a bending or strain the cantilever which is measured as a simple resistance change. The volumetric shift in the sensing material may be due to diffusion of the analyte molecules into the sensing material, probe-target binding on the material surface or bulk, or surface or bulk chemical reactions between the analyte and sensing material. Cantilever strains of only a few angstroms are potentially measurable in many cases.

In the past, the basic EPM sensor platform has been used with a variety of sensing materials in various applications. These sensing applications include volatile organic compounds or VOC's [3, 4, 6], in which multiple polymer sensors are used in an array or artificial nose. Here, a given VOC is expected to produce a unique signature within the sensor array. Poison gases including hydrogen cyanide and organophosphate vapors [8-12] have also been tested. In these cases, a polymer host matrix may be utilized to immobilize a chemical probe species such as functionalized gold nanoparticles or biological molecules such as proteins that are sensitive to the organophosphate nerve agents. Dangerous industrial compounds including hydrogen fluoride [13], environmental toxins such as carbon tetrachloride, trichloroethylene, and estrogen in water [14] have also been tested. In the case of estrogen, biological molecules (estrogen antibody) immobilized in a host matrix provide the sensing material. Biological molecules have also been detected as analytes in both liquid and aerosol phase. These include viruses such as vaccinia virus, an analogue to smallpox, [15], and various bacteria [16]. In these cases, antibodies for the analyte antigen were used within the sensing material as probes. A single-strand DNA sensor was also tested previously, using the thiolated complimentary strand immobilized on a gold surface as a probe [17]. Medical applications are also currently being tested for the basic sensor platform. A small intraoral sensor designed to give a quick, non-invasive measurement of human hydration state [18, 19] is currently being developed for potential commercial applications such as military testing of soldiers in hot environments, pediatric hydration testing, geriatric testing applications and use in sports hydration monitoring [20]. These hydration applications rely on a propriety hydrogel responsive to osmolality as a sensing material [19]. Custom sensing materials that integrate total neutron exposure have also been recently tested as devices for monitoring the presence of radioactive, neutron producing sources. These devices utilize boron oxide containing composite materials to integrate total neutron exposure.

In the current application, we have developed EPM sensors to detect the presence of methyl salicylate, or MeS. MeS is an organic ester, produced in nature by a variety of plant species. MeS is also known by the common name oil of wintergreen, and is used in industry as a flavoring or fragrance in many consumer products. In addition to fragrance or flavoring applications, MeS is also commonly used for the simulation of the poisonous mustard gas.

Mustard gases, also known as sulfur mustards, are colorless liquids at room temperature in pure form. In vapor phase, exposure to these agents causes skin blistering and internal lung blistering or damage when inhaled. These agents were produced and used during World War One in large quantities. MeS is also used in various industrial applications as a chemical tracing agent. In these applications, the MeS vapor is used as a marker to test the flow of vapors in and around fittings, channels and other avenues of vapor flow in specific devices and products. For example, MeS is commonly used as a tracer in the design and testing of protective suits worn by soldiers, fire personnel or police in order to protect them from dangerous external agents in vapor form. In applications such as protective suit design, it is important to be able to detect the presence of the tracer vapors at multiple locations within the protective suit in real time. EPM sensors are tiny sensors resistant to movement or shock, and require only very simple electronics to operate. Here, we have fabricated two potential EPM sensors capable of detecting MeS vapors. In the most simple design, we have used the common organic polymer, poly (ethylene vinyl acetate), or PEVA. The Hildebrand solubility parameter of PEVA is 18. 6 $MPa^{1/2}$, relatively close to the solubility parameter of methyl salicylate, 21. 7 $MPa^{1/2}$. In this design, we do not expect a large specificity from a single polymer-based sensor. A sensor of this type would only be viable in an application where a single known analyte, in this case MeS, is used. This first sensor would rely on the solubility parameter match between

MeS and PEVA so that the MeS molecules would spontaneously partition into the PEVA volume [21], resulting in a tiny volumetric change in the PEVA that could be detected by the microcantilever embedded in or in physical contact with the PEVA material. It is also expected that this first sensor would be reversible. Here, if the analyte is removed, we would expect that the analyte molecules would slowly diffuse out of the sensing material, restoring the sensor to its original configuration or resistance.

In the second potential MeS design, we have used a composite material consisting of a bio-compatible host hydrogel matrix (Hypol) functionalized with an enzyme that is highly specific to methyl salicylate (SA-binding protein 2, or SABP-2). Hypol is produced by Dow Chemical, and has been shown to preserve the bio-activity of a variety of biomolecules that are immobilized in its bulk or on its surface. This gel is cured upon exposure to water, either in the vapor phase or as a liquid. Vapor-phase curing generally takes place over a 24-hour period, while liquid-phase curing can be occur in as little as a few minutes. SABP-2 is a 29 kDa protein that catalyzes the conversion of methyl salicylic acid into salicylic acid in many plants. Here, the enzyme was extracted from the tobacco plant [22] and purified in a buffer solution. In this study, it is hoped that a reaction with the bio-active SABP-2 and the MeS analyte will result in some type of volumetric or conformational change in the tiny composite sensing material. It might also be assumed that most of the reactions taking place will be on or near the surface region of the composite sensing material. This change would then result in a bending of the microcantilever, or a change in the pre-loaded microcantilever that would indicate the presence of the analyte. For this second type of sensor, we might expect that the detection reaction would be irreversible. Once the analyte is removed, there will be no reversal of the reactions that initially took place between the enzyme molecules and the MeS analyte. This type of sensor may be referred to as a chemical fuse. We might expect, though, that multiple exposures of this type of sensor to the analyte, especially at low exposure levels, will be possible. After many exposures, though, the sensor may ultimately become fully saturated. This sensor, then, acts as an integrator, measuring over multiple exposures the total analyte dose. In this report, we present the results of preliminary testing of the two candidate MeS sensors to nominal exposures to MeS in the vapor phase.

2. Experimental

The individual, non-functionalized piezoresistive microcantilevers were produced by Cantimer, Inc., Menlo Park, CA. These cantilevers are approximately 150 microns in length, and 40 microns wide, with a nominal resistance of 2. 1–2.3 k Ohms (Fig. 1).

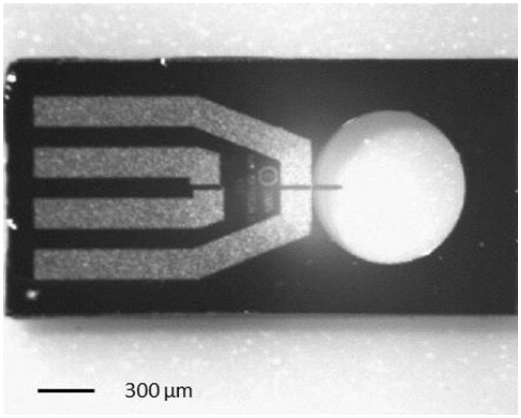

Fig. 1. Micrograph of a microcantilever die. The microcantilever extends into a small circular area in order to contain the sensing material and also to partially protect the cantilever. Each die also contains an integrated thermistor for applications where temperature correction is needed.

Within each semiconductor die, the cantilever extends into a small circular area in order to contain the sensing material and also to partially protect the cantilever. In addition, each cantilever die assembly contains an integrated thermistor if temperature correction is needed. For short duration experiments where temperature correction is not needed, this thermistor is not connected or monitored. Each sensor-containing die is then attached to a suitable carrier assembly, including a connector for electrical connections to the cantilever or thermistor. For the current MeS experiments, we used a single-chip AD7792 24-bit A-D converter which functions as a 6 ½ digit multimeter to directly measure the cantilever resistance using only two leads. The AD7792 also contains two integrated precision current sources. These are used as excitation for the cantilever or thermistor. Here, the excitation current used was 200 µA. The 24-bit converter then measures the voltage drop across the cantilever or thermistor in order to make a measurement. Any bending of the cantilever thus results in a small change in the measured voltage drop across the cantilever for a given constant excitation current. The AD7792 chip is interfaced to a laptop computer through a USB interface provided by an USBmicro 421 chip [8, 23]. The user interface and data collection was performed on a laptop computer using a custom LabView interface instrument. As mentioned previously, this same electrical setup may be easily interfaced to commercially available radio transmitters in order to deploy multiple sensors in a mesh network of connected sensors [9].

The PEVA-based sensors were fabricated from PEVA polymer obtained from Aldrich. The molecular weight of the polymer used was approximately 100 k. This polymer was dissolved in toluene to form a viscous liquid. Two types of PEVA-based sensors were fabricated from this PEVA solution. Small sensing material bead sensors

were fabricated by physically making contact with the topside of the microcantilever near the cantilever end with the PEVA solution. A tiny volume of PEVA was lifted from the PEVA liquid, attached to the cantilever end. We estimate the volume of this sensing material bead to be only approximately 1 µl. This bead was allowed to dry for 24 h, and the subsequent assembly was lowered onto a Si substrate until the PEVA bead began to make contact with the substrate. This pre-loading of the microcantilever continued until the cantilever bending reached a value of 50 Ω. The full assembly was then cemented in place with epoxy to form a single, rigid sensor (Fig. 2).

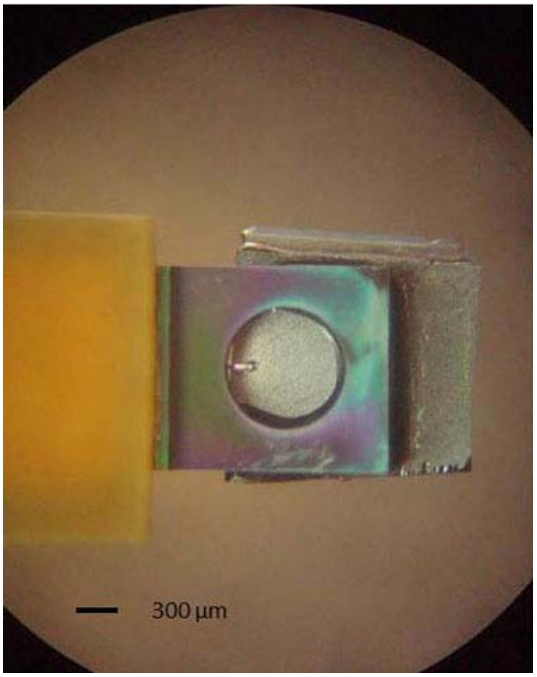

Fig. 2. Micrograph of a functional small PEVA MeS sensor. The piezoresistive microcantilever is pre-loaded onto a tiny bead of PEVA sensing material. Electrical traces are not visible in this micrograph.

For the second type of PEVA sensor, a large bead of sensing material was used. Here, the viscous PEVA solution was directly deposited onto the bottom Si substrate and allowed to dry for 24 h. The estimated volume of this large sensing material bead is 10-20 µl. After drying, a micromanipulator was used to gently bring the microcantilever into contact with the top surface of the sensing bead. Again, the microcantilever was pre-loaded by 50 Ω equivalent bending and the assembly was cemented in place.

For the Hypol-SABP-2 composite sensing material, concentrated and purified SABP-2 from tobacco plant in HEPES buffer solution was generously donated by Professor Dhirendra Kumar at East Tennessee State University. The concentration of the SABP-2 was approximately 2 µg/ml of buffer solution. For the composite sensing material, approximately 20 µl of liquid Hypol, and 20 µl of SABP-2 solution were used. These materials were mixed together rapidly and vortexed for 30 s. This results in approximately 0. 040 µg of active SABP-2 in the full material sample after curing. Sensing material beads of approximately 20 µl volume were quickly deposited onto pre-prepared Si substrates and allowed to cure for 24 h. After curing, a micromanipulator was used to gently bring the microcantilevers into contact with the top surface of the sensing beads. Again, the microcantilever was pre-loaded by 50 Ω equivalent bending and the entire assembly was cemented into a single rigid unit.

Exposures to MeS were performed in a small, plexiglass test chamber. Two exposure levels were used for these sensors. For large MeS exposures, the level used was approximately 150 ppm. This value is very close to the equilibrium vapor pressure for MeS at 21 ^{o}C, which is 160 ppm. For the smaller exposure levels, the value was approximately 30 ppm. Both the temperature and the humidity were kept constant throughout the short MeS exposures. In Fig. 3, two fully assembled PEVA sensors are shown. The sensor dies are attached to 10-pin micro connectors on 3-inch long flexible circuit boards. All electrical connections are made to the 10-pin connectors. For these experiments, only two connections for the cantilever resistance (voltage drop) were needed.

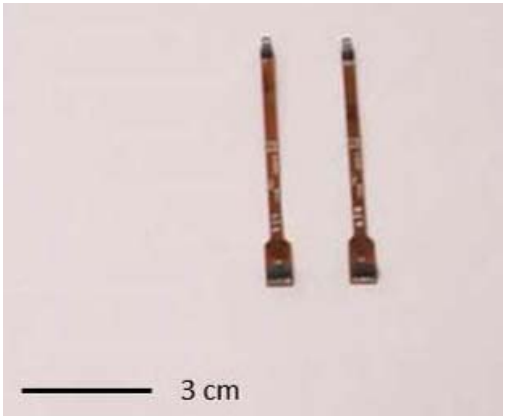

Fig. 3. Two fully assembled PEVA sensors. The sensor dies are attached to 10-pin micro connectors on 3-inch long flexible circuit boards.

3. Results and Discussion

In Fig. 4, we indicate the results of the small sensing material bead PEVA sensor to 150 ppm of MeS at 20 ^{o}C. Arrows on the figure indicate the times where MeS was first introduced into the test chamber, then removed from the chamber, then re-introduced into the chamber, then finally removed again. Initial sensor response to the introduction of the MeS was generally rapid, with the sensor beginning to indicate the presence of MeS within about 5 s of exposure. Also, once the MeS is

removed, the sensor begins to recover from the exposure within 10 – 20 s. In both exposures indicated in Fig. 4, the sensor was not allowed to fully saturate prior to the MeS being removed from the chamber, however in the second exposure some initial signs of saturation were beginning to be indicated.

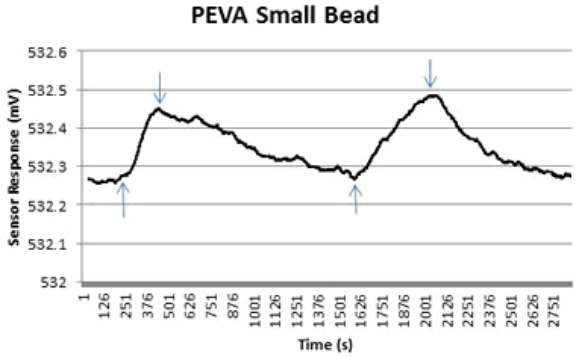

Fig. 4. Results of the small sensing material bead PEVA sensor to 150 ppm of MeS at 20 ^{o}C. Two full cycles of exposure followed by analyte removal from the test chamber are indicated.

After the first MeS exposure, nearly 20 min elapsed before the sensor was able to recover to its original resistance value, while after the second full MeS exposure, the sensor recovered to its original state after approximately 12.5 min. This may indicate some sensor "conditioning" owing to the previous exposure is occurring. The approximate full sensor response of 0.2 mV corresponds to a microcantilever resistance change of approximately 1 Ω. The nominal deflection sensitivity for our microcantilevers is approximately 4.7 Ω/μm. Thus, the physical strain of the microcantilever indicated in Fig. 4 is approximately 0.21 μm. Exposing the large bead PEVA sensor to 150 ppm of MeS is indicated in Fig. 5.

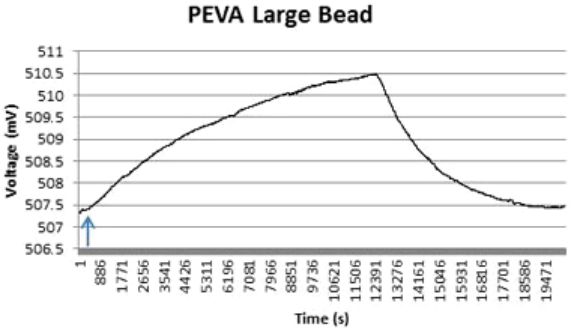

Fig. 5. Exposure of the large bead PEVA sensor to 150 ppm of MeS, followed by removal of MeS from the test chamber.

The total exposure time for this test was approximately 200 min. Near the end of this lengthy exposure, the sensor began to indicate saturation to the presence of the analyte. The total resistance change for the microcantilever is 15 Ω, corresponding to a cantilever strain of approximately 3.2 μm. After the MeS is removed, the sensor returns to its original resistance value over approximately 140 min. The slow sensor response for this large bead sensor accompanied by the overall large total resistance change is expected for this type of sensor. Generally, the large sensing material bead produces an overall larger volume change and thus a larger cantilever deflection when compared to the small bead sensor. The volume change in the sensing materials for the PEVA-based sensors is due to the partitioning of the analyte molecules into the sensing material bulk. For the majority of materials such as PEVA used in EPM sensors, models of "anomalous" gel swelling most accurately match experimental data, such as in this case [24]. In these anomalous diffusion models, analyte uptake results in an intermediate type of solvent molecule transport, involving portions of both standard Fickian diffusion and time-independent diffusion where the swelling depends linearly with exposure time [24].

In order to obtain full or nearly full saturation of analyte molecules in the sensing material, we expect that large volume sensing materials will simply require more exposure time to reach this point. With regard to applications in which real-time monitoring of MeS vapor is needed, the small bead sensors based on PEVA generally result in faster time responses and recoveries. In future studies, it may be possible to further reduce the PEVA sensing material volume so as to provide even faster response and recovery times.

In Fig. 6, the SABP-2 sensor results for exposure to MeS at 150 ppm are indicated.

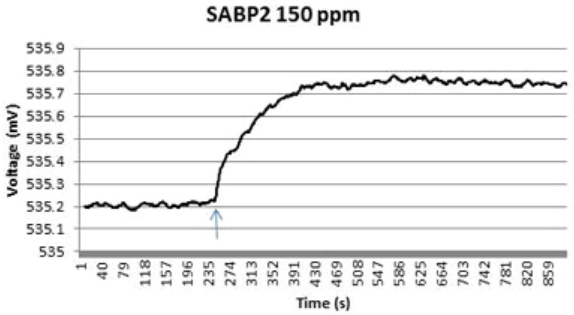

Fig. 6. SABP-2 Hypol composite sensor results for exposure to MeS at 150 ppm. Initial exposure to MeS is indicated by the arrow on the chart. Here, we see that the sensor response to the analyte is almost immediate, with a large and rapid change in microcantilever resistance. Full saturation of the sensor is achieved within 150 s of MeS exposure. This form of MeS sensor does not recover, instead operating as a chemical fuse or integrator of total MeS dose.

Initial exposure to MeS is indicated by the arrow on the chart. Here, we see that the sensor response to the analyte is almost immediate, with a large and rapid change in microcantilever resistance. Full saturation of the sensor is achieved within 150 s of MeS exposure. The total resistance change of the microcantilever is 2.75 Ω, corresponding to a cantilever deflection or strain of 0.58 μm. It has been shown that SABP-2 has strong esterase activity with methyl salicylate as the substrate, and that salicylic acid is a potent product inhibitor of this catalysis [25]. These results suggested that in plants SABP-2 may convert MeS to salicylic acid as part of the signal transduction pathways that activate systemic acquired resistance. In the current application, the precise reaction of the MeS molecules with active sites on the SABP-2 enzyme is unknown; however a rapid and measurable reaction resulting in an overall volumetric shift in the sensing material is clearly evident. After removal of the analyte from the test chamber (not shown), the saturated sensor did not recover to its original resistance value, remaining instead at its fully exposed and saturated resistance state. This type of sensor behavior is generally referred to as that of a chemical fuse, giving only the initial indication of the presence of an analyte. These sensors would, however, be capable of identifying multiple exposures to the analyte as long as the integrated sensor exposure remained below the full sensor saturation level.

The SABP-2 Hypol composite sensors were also exposed to low levels of MeS, at approximately 30 ppm. Fig. 7 shows the sensor response to this level of MeS, with initial exposure at time 100 s. As with the 150 ppm exposure using the SABP-2 composite sensing material, the initial sensor response is almost immediate. Exposure to the MeS vapor occurred for over 30 min, however total sensor saturation was not achieved. Total resistance change for this exposure period was 1.25 Ω, and calculated microcantilever deflection was 0.26 μm. For this resistance change and cantilever deflection value, we do not expect to observe sensor saturation when compared to the previous result for 150 ppm MeS exposure. Instead, the sensor continues to react with the MeS over time, integrating a total dose value.

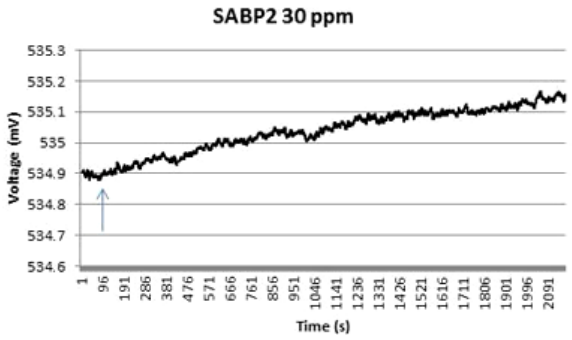

Fig. 7. Low exposure to MeS for SABP-2 Hypol composite sensor. Arrow indicates time of exposure. Exposure was approximately 30 ppm maximum.

We have previously used hydrogels or other polymers to immobilize biological molecules as probes in EPM sensor platforms. In one experiment, the enzyme acetylcholinesterase (AChE) was used as a probe immobilized in Hypol. This sensor was successfully tested for detection of the organophosphate diisopropylfluorophosphate, or DFP [10]. The bacteria bacillus subtilis was also detected in an aerosol delivery utilizing a sensing material formed by immobilizing the antibody, anti-*Bacillus subtilis*, in a Hypol host [10]. The chemical estrogen was detected using anti-estrogen in a compound sensing material [14]. In these cases, including the present study, it is not determined physically where the biological reactions are taking place within the sensing material. While gels such as Hypol are very porous materials, it is difficult to envision large molecular weight biological target analyte molecules diffusing deeply into the bulk of the host matrix. In all of these cases, we expect that the vast majority of the binding reactions taking place are at or near the surface regions of the sensing materials. It is not, therefore, expected that sensor response as a function of sensing material volume changes will follow the same model behavior, characterized by partitioning or diffusion, exhibited by more common polymeric sensing materials. Further studies underway with the current MeS sensing materials includes altering the ratio of bio-probe molecules to the host material, as well as measuring the speed and total response of the sensors as a function of the sensing material starting volume.

4. Conclusions

MeS detection capable sensors utilizing the basic EPM sensor platform have been fabricated and tested. Sensing materials included the polymer PEVA and a composite material consisting of SABP-2 immobilized within a Hypol matrix. The PEVA-based sensors exhibited slower but reversible responses to MeS vapors, recovering fully to their initial state after the analyte was removed. SABP-2 sensors exhibited faster overall response to the introduction of MeS, responding nearly instantly. These sensors, however, do not recover after exposures have ended. Sensors using the SABP-2 sensing materials act instead as integrating sensors, measuring irreversibly the total MeS dose obtained.

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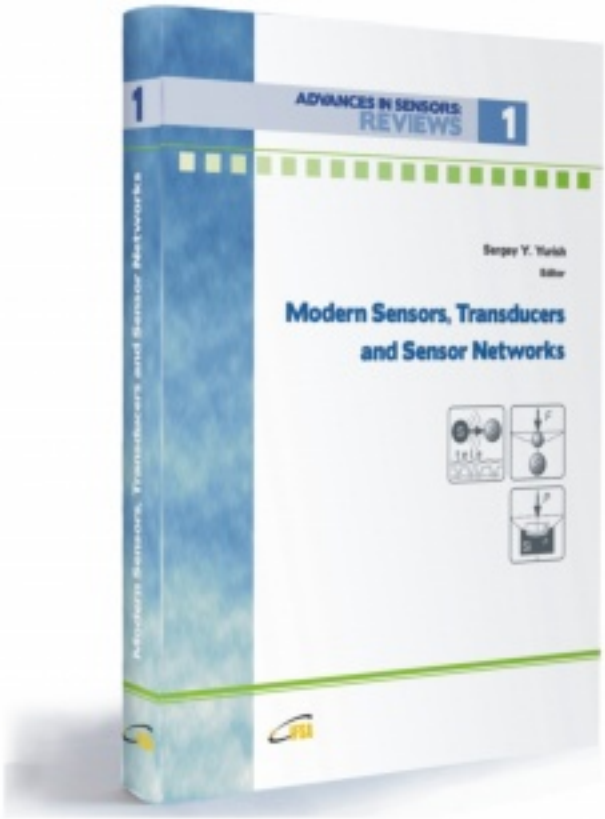

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