

Ethanol vapor detection in saline solution using piezoresistive microcantilevers

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We report detection of ethanol in a phosphate buffered saline (PBS) solution using a low-power piezoresistive microcantilever-based system that has the potential to be used in the human body. PBS was used to simulate interstitial fluid and a permeable hydrophobic membrane was employed to transport ethanol vapor to the sensor while blocking the liquid and ions of the PBS. Commercial gold-coated cantilevers were functionalized with polymers for optimal ethanol response. Advantages of this device are its low-power consumption, its high sensitivity, and its capabilities for miniaturization into an implantable capsule. The limit of detection for ethanol in PBS was found to be less than 100 ppm or 8 mg/dl. © 2006 American Institute of Physics.

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I. INTRODUCTION

Ethanol detection is important for societal and industrial applications and for physiological studies on alcoholism. For the most precise measurements, high performance liquid chromatography (HPLC) and infrared spectroscopy can be used for ethanol concentration reference measurements, but these involve expensive and large equipment. For less sensitive detection, smaller handheld devices such as breathalyzers have been used for measurements that are proportional to blood alcohol concentration (BAC). These devices acquire ethanol from exhaled breath. The breath alcohol can be difficult to correlate to BAC since there can be so much variation in the breath collection method. Many such sensors rely on electrochemical techniques wherein an electric current is produced during alcohol oxidation. Some sensors for physiologically based detection use enzymes such as alcohol dehydrogenase (ADH) which metabolizes ethanol by oxidizing it to acetaldehyde.¹ Although enzymes are highly selective, degeneration and reversibility over time become an issue.

To miniaturize systems and decrease costs, microelectromechanical systems (MEMSs) such as microcantilevers and microcapacitors have been developed. Microcantilevers, originally developed for scanning probe systems, can be used as transducers when their bending is attributed to surface stresses induced by specific interactions with the environment. Microcantilever deflection changes as a function of surface stress. Using Stoney's formula and the equations for bending of a cantilever, a relation can be derived between the bending and the changes in surface stress. The surface stress variation between top and bottom surface of a cantilever can be written as^{2,3}

$$\sigma_1 - \sigma_2 = \frac{zEt^2}{3L^2(1-\nu)}, \quad (1)$$

where z is the cantilever deflection at its free end, E is Young's modulus, L is the cantilever length, t is the thickness, and ν is Poisson's ratio. Since all the quantities on the right hand side can be measured (or are known *a priori*), the changes in surface stress due to adsorption can be calculated. Deflections of microcantilevers caused by induced surface stress have been detected using optical readout techniques since the discovery that metal-coated microcantilevers are affected by temperature and humidity.⁴ These microcantilevers have been used in gaseous and in fluid states to identify small quantities of analytes.⁵⁻⁹ However, an optical system requires laser alignment which encompasses a larger footprint and has relatively higher power requirements. Piezoelectric microcantilever sensors have also been used,¹⁰ but sensitive measurements then require the detection of resonance shifts (rather than bending) due to the relatively large thickness of the zinc oxide piezoelectric layer on these microcantilevers. Resonance detection requires considerable additional electronics and is far too complex for implantable sensors.

To miniaturize chemical detection systems while maintaining high sensitivity, either chemicapacitive sensors or piezoresistive microcantilevers can be used. Chemicapacitive sensors have been used to detect volatile organic compounds.¹¹ They provide a low-cost, low-power alternative to larger systems and offer high sensitivity. They rely on changes in permittivity of a polymer to directly detect ethanol and other volatiles. In general, the drawbacks of chemicapacitors are that the compound of interest must cause a significant increase in the dielectric constant and chemicapacitors endure a high leakage when placed in an ionic fluid.

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However, they were recently successfully used in aqueous solution by using a vapor permeable, hydrophobic membrane.¹²

Piezoresistive microcantilevers were first used for miniaturization in atomic force microscopy by Tortonese *et al.*¹³ Changes in the surface stress of cantilevers can cause bending which translates to a resistance change. Piezoresistive microcantilevers have demonstrated sensitivity to humidity, explosives, and temperature due to laser irradiation.^{14,15} Piezoresistive microcantilevers with a coating of about 10 μm photoresist, a light sensitive polymer, also have been used to measure alcohol in the gaseous state where a sensitivity of less than 10 ppm was achieved.¹⁶ However, the experiment was not conducted in a humid environment due to the complicating factor of ethanol-water interactions and the sensitivity of the microcantilevers to water. Piezoresistive microcantilevers coated with an UV sensitive polymer have been dipped in a liquid environment to measure ethanol in water, but the alcohol content was very high.¹⁴ The well-known affinity of ethanol and water for one another makes it difficult to separate small quantities of ethanol in water or vice versa. For example, simple fractional distillation can only attain 95.6% ethanol. For physiological measurements, a BAC sensitivity of 0.01% is needed for significant detection in a humid environment.

In this article we present a very low-power and compact system based on functionalized piezoresistive microcantilevers that has the potential to be used to detect ethanol in the human body. The affinity of ethanol and water for one another makes this task more difficult and requires both filtering and sensing. Details of the hydrophobic, vapor permeable membrane used to overcome interactions with various ions present in the human body and to achieve operation of the device in a liquid environment are given. The electronics used to process the obtained signal are presented with the future objective of combining the electronics with a telemetry system inside an implantable capsule. The selection and coating process for various polymers used to functionalize the gold-coated microcantilevers for the purpose of optimizing the sensitivity and selectivity of the sensor device is also described. Data are presented at both room temperature and at a slightly elevated body temperature. The limit of detection (LOD) of the system is found to be less than 100 ppm by volume ethanol in phosphate buffered saline (PBS), which corresponds to a BAC level of 0.008%, or 8 mg/dl.

II. EXPERIMENTAL SETUP

The experimental setup, illustrated in Fig. 1, includes an open beaker with PBS solution, a partially submerged chamber with a hydrophobic membrane to allow volatile substances into the chamber while impeding liquids, a pipet to introduce ethanol to the solution, a water bath, and a hotplate to simulate human body temperature. The chamber contains microcantilever piezoresistive sensors placed in a half-bridge configuration and electronics that process the differential signal from the bridge.

The active sensor utilizes piezoresistive microcantilever arrays (CantiTM Chip 4) manufactured by Cantion, Inc.

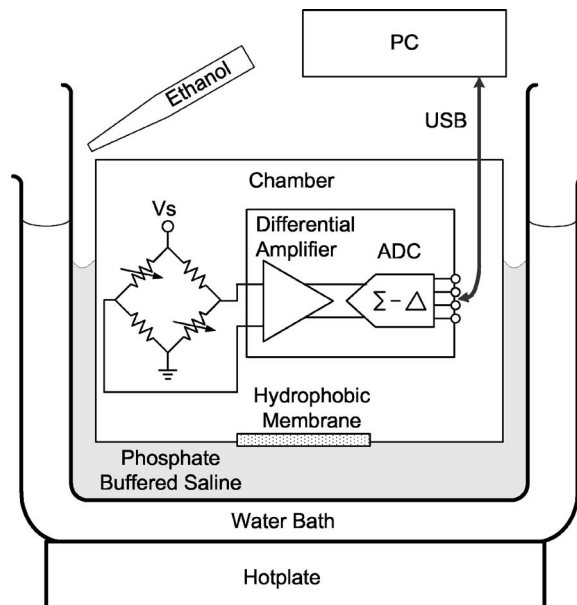


FIG. 1. Schematic of the experimental setup. The active elements of the Wheatstone bridge are microcantilevers. The ADC processes the signal from the bridge and outputs a 16 bit number to the computer.

(Lyngby, Denmark).¹⁷ The four-cantilever array is fabricated with uniformly linearly doped (*p*-type) silicon “wires” encapsulated in silicon nitride (Si_3N_4). The resulting embedded 4 k Ω resistive wire is laid down in a loop with connections to two bond pads for each cantilever. However, one of the pads of each cantilever is tied to a common electrical connection, somewhat limiting the number of different connection combinations. In this study, two cantilever arrays were required to configure the half-bridge in Fig. 1, where one gold-coated cantilever was selected from each array. Each array consisted of two bare Si_3N_4 cantilevers and two cantilevers coated with a 2 nm chromium (Cr) adhesion layer overlaid with 30 nm gold (Au) by vacuum physical vapor deposition. The cantilevers are 50 μm wide, 120 μm long, and 0.5 μm thick.

A diagonal-type Wheatstone half-bridge consisting of two Au-coated cantilevers along with balancing resistors proved to be the best configuration for our purposes. The bridge typically requires approximately 1 V, V_s , for adequate signal levels to be delivered to the AD7792 chip by Analog Devices (Norwood, MA).¹⁸ Inherent noise arising from thermally induced motion of the sensor and minor shot noise are also picked up by the device. This very low-power chip consists of a differential amplifier and a 16 bit sigma-delta analog-to-digital converter (ADC). Thus the differential bridge voltage as a function of cantilever resistance may be converted to a serial data stream. A USB data acquisition board is used to interface the digital signal to the computer. A 3 V lithium battery powered the circuit to eliminate 60 Hz line noise.

The electronic board incorporating the cantilever sensors was placed in a miniature plastic cup with a hydrophobic, vapor permeable nanopore membrane sealed to the bottom. The hydrophobic membrane allows vapor to pass while blocking liquid, thus exposing the sensors only to ethanol

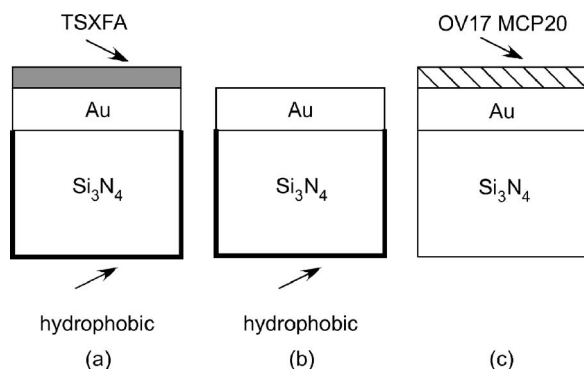


FIG. 2. Schematic of cantilever cross sections showing different functionalizations (not to scale). In (a), a hydrophobic coating is silitated to the Si₃N₄ surfaces, and TSXFA is thiolated on the Au surface. In (b), only the hydrophobic coating is silitated to the Si₃N₄ surfaces with no coating on the Au surface. In (c), OV17 MCP20 is thiolated on the Au surface.

and other volatiles. For this experiment, we used the General Electric Osmonics, Inc. (Minnetonka, MN) nylon membrane, which measures 13 mm in diameter with a 0.1 μm pore size.¹⁹ The membrane does not allow fluid to pass through the nanopores at pressures less than 35 psi.¹⁹ The membrane surface on the sensor side and the inside of the cup were observed immediately following the experiment, and no fluid was seen in the system or on the membrane surface. The membrane holder was sealed to the cup with clear silicone sealant around its edges. The cup was placed in a glass beaker of 100 or 200 ml of PBS solution with a 7.4 pH. The experiments were conducted at room temperature (22–23 °C), with the exception of one set of measurements where the glass beaker was placed in a water bath heated to 39 °C. Data were transmitted to the computer at a sample rate of 16.7 Hz with a digital gain of 128.

III. SENSOR FUNCTIONALIZATION

The microcantilevers were functionalized by three different methods for transducer evaluation. The polymer coatings were carefully selected based on their high partition coefficients for ethanol.¹² For each bridge, two Au-coated cantilevers were identically chemically treated.

The first set of cantilevers, shown in Fig. 2(a), was coated with a 4% solution of 13-(chlorodimethylsilylmethyl) heptacosane 95% in hexane in a dry nitrogen environment and left for 24 h. The cantilever arrays were then rinsed in ethanol and dried with nitrogen. The 13-(chlorodimethylsilylmethyl) heptacosane 95%, purchased from Gelest, Inc. (Morrisville, PA), created a hydrophobic coating by silitation on the Si₃N₄ surfaces of the cantilevers.²⁰ After applying this hydrophobic coating, the arrays were coated by dipping them into a 10% solution of thiolated siloxane fluoro alcohol (TSXFA) in ethanol that was provided by Seacoast Science, Inc. (Carlsbad, CA).²¹ The cantilevers were again left in a humid environment overnight and then rinsed in ethanol and nitrogen dried. The TSXFA attaches to the gold surfaces on the cantilevers via the sulfur bond in the thiol to form a self-assembled monolayer.

The functionalization of the second set of cantilevers, shown in Fig. 2(b), entailed dipping the cantilevers in a 3%

solution of 13-(chlorodimethylsilylmethyl) heptacosane 95% in hexane in a dry nitrogen environment and left for 24 h. The cantilever arrays were then rinsed in ethanol and dried with nitrogen. No coating was applied to the Au surface.

The third functionalization, shown in Fig. 2(c), involved dipping the cantilevers in a 5% solution of methyl phenyl mercapto propyl silicone (OV17 MCP20) in toluene that was also provided by Seacoast Science, Inc. The cantilevers were left in a humid environment for 23 h and then rinsed in ethanol and nitrogen dried. The OV17 MCP20 also attaches to the gold surfaces on the cantilevers via the sulfur bond to form a self-assembled monolayer. No hydrophobic coating was used with this set of cantilevers.

IV. RESULTS AND DISCUSSION

To optimize the sensor sensitivity, three different coating schemes were used. The aim of the hydrophobic coating in the first scheme, as seen in Fig. 2(a), was to minimize the interactions of water and ethanol with the Si₃N₄ surfaces. Since both water and ethanol are polar compounds, the hydrophobic coating should not be affected by water or ethanol. The hydrophobic coating was used on the backside of the cantilevers to maximize the bending response of the cantilevers by isolating interactions to only one side of the cantilevers. The polymer TSXFA was chosen to coat the Au surface because its partition coefficient for ethanol is about three times that for water.¹² A control experiment was performed with just the hydrophobic coating in the second scheme of coatings. The third set of data used a second polymer, OV17 MCP20, which also had a higher measured partition coefficient for ethanol than water, although the partition coefficient for ethanol is about half the ethanol partition coefficient for TSXFA.¹² The sensor response to the controlled addition of ethanol to the PBS solution with each of the three different cantilever coatings is discussed and compared. Both short term and long term effects were investigated.

Piezoresistive microcantilevers coated with a hydrophobic compound on Si₃N₄ (backside) and the polymer TSXFA on the gold side (topside) of the cantilevers were used to take data at room temperature. The aim of using both coatings was to have the two coatings work in conjunction to improve sensitivity. The container consisting of the cantilevers and electronics was placed in 100 ml of PBS solution. After the sensor stabilized from electronic heating and initial water vapor introduction, 300 ppm by volume ethanol was added to the solution, as seen in Fig. 3. The sensor response was immediate. Since the liquid container is open to the environment, the signal returns to its base line in about 10 min due to evaporation. A subsequent dose of 1000 ppm by volume ethanol was pipetted into the solution where the peak height is three times the peak height for the 300 ppm ethanol introduction. For a control measurement, 1000 ppm water was injected into the solution, and no signal response was seen. Although the hydrophobic/TSXFA coated cantilevers gave a good response, additional measurements two days later did not yield the same sensitivity, indicating that degradation of one or both of the coatings may be an issue.

Measurements using piezoresistive microcantilevers

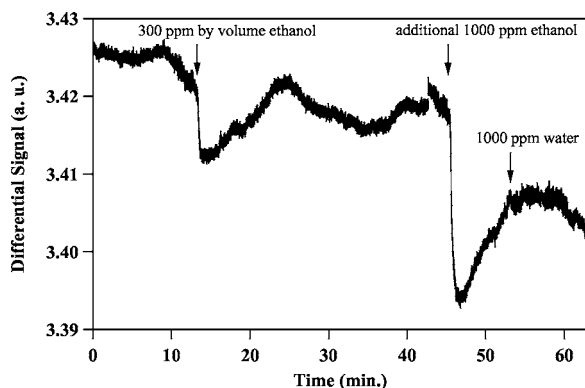


FIG. 3. Hydrophobic/TSXFA coated sensor output vs time. Measurements were taken at room temperature using piezoresistive microcantilevers coated with a hydrophobic coating and the polymer TSXFA. At $t=13.2$ min, 300 ppm by volume ethanol was added into a PBS solution. At $t=45.3$ min, an additional 1000 ppm by volume ethanol was pipetted into the PBS solution. At $t=51.2$ min, 1000 ppm by volume water was injected into the solution.

coated with the hydrophobic layer, 13-(chlorodimethylsilyl-methyl) heptacosane 95%, were taken at room temperature in 200 ml of PBS solution. 150 ppm by volume ethanol was introduced to the solution, as seen in Fig. 4. Again, the ethanol signal returned to the base line in about 10 min. The base line has a decreasing slope due to the ever-present water vapor. The response of the sensor typically flattens out when water equilibration is achieved, but this occurs on a longer time scale of the order of hours. This sensor, with Au on one side and a hydrophobic coating on the Si_3N_4 side, appears to be sensitive to ethanol and water vapor. In a previous control experiment, a sensor with Au on one side and bare Si_3N_4 on the backside gave no response to ethanol. Thus, we believe that the hydrophobic coating is responsible for the change in signal. Preliminary quartz crystal microbalance (QCM) measurements taken with the hydrophobic coating to determine partition coefficients for ethanol and water support these data. The hydrophobic coating was refluxed at 50 °C for 24 h, and additional QCM measurements were taken that still showed some sensitivity to ethanol and water.

The signal response to ethanol in the hydrophobic/

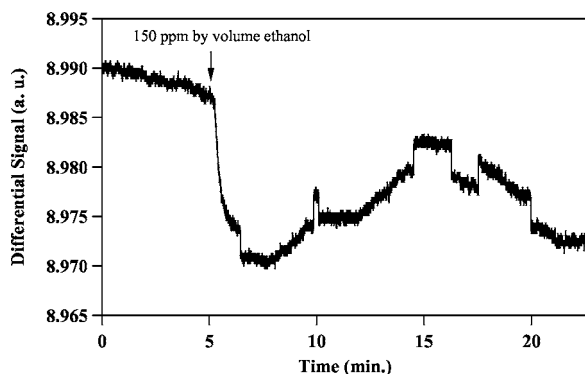


FIG. 4. Hydrophobic coated sensor readings vs time. Measurements were taken at room temperature using piezoresistive microcantilevers coated with a hydrophobic coating. At $t=6.1$ min, 150 ppm by volume ethanol was injected into a PBS solution.

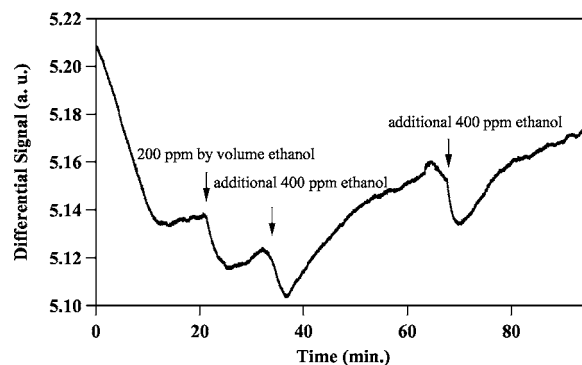


FIG. 5. OV17 MCP20 coated sensor output vs time. Measurements were taken at room temperature using piezoresistive microcantilevers coated with the polymer OV17 MCP20. At $t=20.9$ min, 200 ppm by volume ethanol was injected into a PBS solution. At $t=34.0$ and $t=67.6$ min, an additional 400 ppm by volume ethanol was injected into the PBS solution.

TSXFA case was not as great as in the case of just the hydrophobic coating. Thus, the two coatings may work against each other instead of in conjunction.

Room temperature measurements using piezoresistive microcantilevers coated with the polymer OV17 MCP20 were taken in 200 ml of PBS solution. The initial base line slope was decreasing due to water introduction. A dose of 200 ppm by volume ethanol was pipetted into the solution, as seen in Fig. 5. Two subsequent doses of 400 ppm by volume ethanol were injected into the solution. Both 400 ppm doses gave corresponding dips in the data. The curve then shows a positive slope following the dips. The ethanol did not completely evaporate before subsequent ethanol doses so the peak was not reached. Therefore, quantitative comparisons cannot be ascertained. In comparison with room temperature measurements with the hydrophobic coatings, the predominant deflection signal for the OV17 MCP20 coated cantilevers is in the opposite direction than the deflection signal using cantilevers coated with a hydrophobic coating. Note that the time scale of the ethanol reaction is also longer (>30 min). The response time for different polymers may be due to the difference in partition coefficients.

Subsequent data were taken with the same OV17 MCP20 coated microcantilevers three days later with the PBS solution sitting in a water bath at 39 °C, as seen in Fig. 6. The gaps in the graph are due to loss in data intermittent between saving data sections. After a small dip in response, the signal again goes in the opposite direction as in the room temperature measurements. The time scale of the ethanol response is 20–30 min. This time scale is consistent with the room temperature measurements since a shorter time scale would be expected at elevated temperature due to a faster evaporation rate of ethanol. As expected, the response is larger than at room temperature due to the higher vaporization of ethanol at elevated temperature. The vapor pressure of ethanol is calculated to be 127.53 mm Hg at 39 °C, which is about 2.5 times the ethanol vapor pressure of 49.48 mm Hg at 22 °C.²² The ethanol signal response at 39 °C is about ten times greater than the response at 22 °C. The polymer partition coefficient for ethanol may also be enhanced at 39 °C. Characterization studies of the polymer

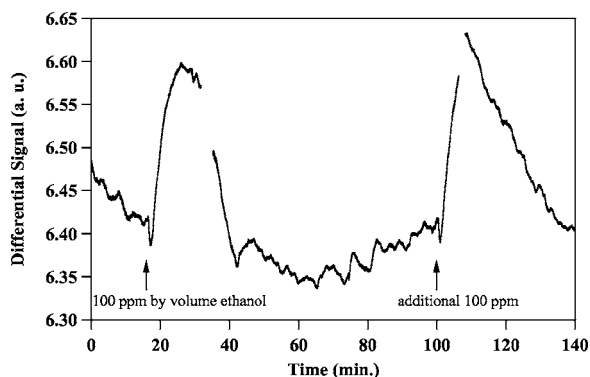


FIG. 6. OV17 MCP20 coated sensor output vs time, taken three days later. Measurements were taken at 39 °C using piezoresistive microcantilevers coated with the polymer OV17 MCP20. At $t=16.5$ min, 100 ppm by volume ethanol was pipetted into a PBS solution. At $t=100.3$ min, an additional 100 ppm by volume ethanol was pipetted into the solution.

using techniques such as surface plasmon resonance and quartz crystal microbalance could provide insight into any polymer changes with temperature.

Ethanol detection was successfully accomplished in a saline solution similar to the interstitial fluid of a human body by a miniature, low-power, microcantilever-based sensor system that effectively separated the ethanol vapor from the liquid. The key to vapor separation was the use of a nanopore hydrophobic membrane.

A number of different sensor coatings were evaluated. Piezoresistive microcantilevers coated with the polymer OV17 MCP20 and microcantilevers coated with the hydrophobic coating gave a similar response at room temperature. Although the hydrophobic/TSXFA coated cantilevers gave a good response, additional measurements of the same sensor did not yield the same sensitivity, indicating that polymer degradation may be an issue. A LOD of less than 100 ppm by volume ethanol was attained where 100 ppm by volume ethanol corresponds to an alcohol concentration of 0.008%. For ethanol detection inside the body, piezoresistive microcantilevers with coatings of OV17 MCP20 and/or a hydrophobic layer can be used. These coatings give a reproducible response to ethanol introduction. More data must be accumulated to ensure that calibration to known ethanol quantities can occur from sensor to sensor. The results of this study show a promising application in detecting blood alcohol concentration *in vivo*.

Selectivity is an important issue for microcantilever sensors. For our purposes we are interested in making an implantable sensor. (The miniature low-power 16 bit sigma-delta ADC chip that was used is capable of interfacing with

a wireless data transfer method and will aid to that endeavor.) There will be few volatile metabolites in the body that will compete with ethanol, but a study of the partition coefficients of certain physiologically significant compounds is needed. In physiological studies or any processes involving a known time course for introduction of ethanol, it is relatively straightforward to correlate the signals with the changes in ethanol concentration so long as the sensitivity is sufficient. Hence, distinguishing among volatiles by chemical means is not necessary. Future experiments will investigate detecting acetaldehyde, a by-product of ethanol metabolism. Acetaldehyde is thought to be in quantities 1000 times less than ethanol in the body, but it is much more volatile than ethanol.

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