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Selective chemosensing and diagnostic breathanalyzer

P. Gouma[a]*, S. Sood[a], M. Stanacevic[b], S. Simon[c]

[a]*Department of Materials science and engineering, State University of New York, Stony Brook, NY*
[b]*Department of Electrical engineering, State University of New York, Stony Brook, NY*
[c]*Department of Biochemistry, State University of New York, Stony Brook, NY*

Abstract

The study of a gas-selective, resistive-type, sensor technology to detect and quantitate nitric oxide (NO) in exhaled breath for diagnostic purposes is described here. The sensor and breathalyzer technology developed by our research group is presented as a tool for the personalized monitoring of the fraction of nitric oxide (FeNO) in exhaled breath, with the long term objective of prevention or control of airway diseases, such as asthma. FeNO is a known biomarker for measuring airway inflammation and it is shown here that the developed technology provides an effective and practical means to quantitate NO levels in breath-simulated samples in a relatively simple and noninvasive way. A sensor microsystem has been developed that quantifies the gas sensor response to generate an accurate measure of the NO concentration in a single exhaled breath.

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Keywords:

1. Introduction

Human breath is composed of nearly 250 gases. Among these, there are certain specific gases, known as “biomarkers”, that are products of human metabolism. Nitric oxide is such a biomarker affecting major biological processes including both focal and systemic inflammation and carcinogenesis and has been extensively studied in the context of pulmonary and cardiac diseases [1]. Moreover, unlike more classical biomarkers which respond to

* Corresponding author. Tel.: +1-631-632-8397;
E-mail address: pelagia-Irene.Gouma@stonybrook.edu

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pathophysiological changes without exhibiting significant biological activity of their own, NO has direct effects on cells and tissues and can contribute significantly to cell and tissue injury. NO is excreted in human airways and is detectable in exhaled air in significant amounts ranging from 0.2–1 ppm in the upper respiratory tract and 1–30 ppm at the nasal level [2-3]. Measuring its fractional concentration in exhaled breath (FENO) provides for a "quantitative, noninvasive, simple, and safe method of measuring airway inflammation that provides a complementary tool to other ways of assessing airway diseases such as infections and asthma" [4]. The potential ability to quantitate NO levels in breath in a relatively simple and noninvasive way would greatly enhance the utility of the technique we describe here for diagnosis and monitoring of a wide range of compromised individuals, including the very elderly, young children, and otherwise incapacitated patients.

NO-targeting diagnostic breath analysis based on NO detection and monitoring remains a challenge, primarily due to the lack of affordable NO detectors of high specificity. The method primarily used to measure exhaled NO is chemiluminescence [3] where the NO contained in a sample reacts with an excess of ozone to produce NO_2 with an electron in an excited state NO_2*reverts back to ground state (NO_2), while releasing electromagnetic radiation in the wavelength of 600–3000 nm range. A photomultiplier tube converts the luminescence in to a readable electrical signal. The technique is highly sensitive and the equipment expensive. Other techniques that can also be used are mass spectrometry and gas chromatography/mass spectrometry.

To-date only a few types of human breath tests have successfully been applied in clinical diagnosis. In 2003, the Food and Drug Administration (FDA) cleared for marketing the first non-invasive test system, NIOX Nitric Oxide Test System [5], made by Aerocrine AB in Sweden, based on chemiluminescence analyzers, to measure the nitric oxide levels in exhaled human breath. The system was intended for use in a hospital since the device, which collects a single-breath sample, has to be connected to a special computer system that performs and displays the results of breath analysis. The Sievers® Nitric Oxide Analyzer (NOA 280i) [6] is another desktop device that measures NO concentrations in exhaled human breath and in liquids. It is used mainly as a research tool.

Both the American Thoracic Society (ATS) and the European Respiratory Society (ERS) have published guidelines for the measurement of FENO, when specific FDA approved devices are used [4]. Currently, there are three devices approved by the FDA for measurement of ***FENO*** in exhaled breath: NIOX® /NIOX® Flex, NIOX MINO® both from Aerocrine, Inc [5], and the InsightTM eNO from Apieron, Inc [6], but they are all suitable for hospital use only. Therefore, there is a need to invest in exploring alternative technologies and additional instrumentation that would facilitate the measurement of FENO at settings other than a hospital or a doctor's office. That is, there is a need for personalized FENO monitoring breathalyzers. The work described here is meant to address this need.

Our group's research has realized an alternative approach to *selective NO detection* in concentrations relevant to those found in human breath and in the presence of interfering compounds relevant to medical applications [7]. Furthermore, handheld, breathalyzer prototypes have been developed and demonstrated by our team recently [8-9]. The path towards the ***diagnostic NO breathalyzer*** is briefly discussed here. Our research group has demonstrated that it is possible to control the microstructure of nanocrystalline metal oxide films and the operating temperature of the sensor so as to employ oxide polymorph phases that are sensitive to only a specific class of gaseous analytes or even be specific to a single species [10-11].

2. A selective nanosensing probe for nitric oxide

Selective NO detection in concentrations relevant to those found in human breath and in the presence of interfering compounds relevant to medical applications has been demonstrated by our research team [12]. A nanosensing probe (was developed based on the γ- phase polymorph of WO_3 (see figure 1), and the ability of the nanosensing probe to detect NO concentrations of interest, that is a few ppm with high sensitivity (that is high amplitude for the change in the resistance of the oxide for a given gas concentration and high specificity i.e., with no cross sensitivity to the presence of potential interfering compounds having the same or higher concentration than that of NO) is demonstrated in Figure 1.

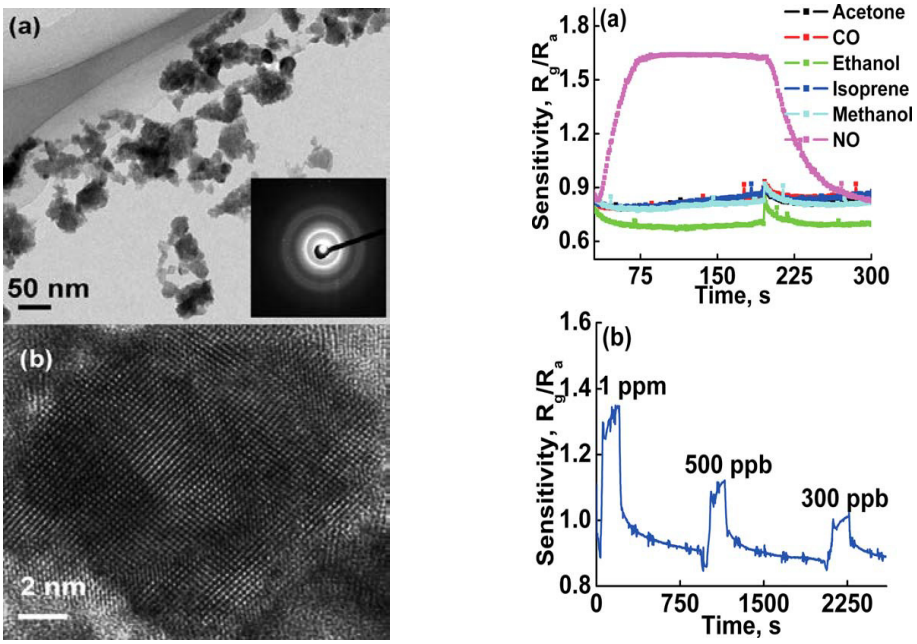

Figure 1: TEM image of as annealed monoclinic γ-WO3. Inset selected area diffraction image showing ring broadening. b HRTEM image of a single monoclinic grain; c) Gas sensing response of monoclinic γ-WO3 to the following gases: 10ppm NO, 10 ppm acetone, 10 ppm isoprene, 50 ppm ethanol, 50 ppm methanol, and 50 ppm CO in a background of 80/20- N2/O2;(d) Low concentration gas sensing response of monoclinic γ-WO3 to 1 ppm, 500 ppb, and 300 ppb NO in a background of 80/20-N2/O2.

Figure 1(c) shows a plot of the sensitivity *Rg/Ra* of the monoclinic nanosensor to 10 ppm of NO at a sensing temperature of 400 °C and compares the NO response of the sensor with the relative response to 10 ppm of acetone and isoprene, 50 ppm of ethanol, methanol, and CO [7]. The sensor responses to the latter gases are manifested as flat lines, meaning no cross sensitivity to these potential interfering vapors was observed. Figure 1(d) shows the sensing response of monoclinic WO_3 sensor to 1 ppm, 500 ppb, and 300 ppb of NO-concentrations that are comparable to those found inhuman breath. The NO nanosensor responds to the presence of the NO gas within seconds giving a clear signal that recovers in the absence of the analyte. The sensing tests were repeated for evaluating reproducibility and the results were always consistent [7].

The sensitivity of cubic ReO_3-type WO_3 toward NO is assumed to be dominated by the adsorption based sensing mechanism that does not affect the bonds on the metal oxide surface. Due to higher oxygen mobility at elevated temperatures or the presence of reducing atmospheres, oxygen vacancies are formed in metal oxides. The slightly reduced metal oxides may be re-oxidized by either pure oxygen or oxidizing gases such as NO_2. Adsorption based sensing mechanism involves incorporation of oxygen in to these vacancies in the presence of an oxidizing gas such as NO_2 or NO.

3. Binary Breathalyzer Prototype

The goal of the first generation breath gas sensor prototype was to provide measurement and quick 'cut- off' response display [8]. For the quick initial pre-screen procedure, the qualitative measurement of the gas concentration is not necessary. LED display is implemented to indicate the binary measurement results. Thus, when the output resistance is higher than a predefined higher threshold, a red LED is lit up; when the output resistance is lower than a pre- defined lower threshold, a green LED is lit up; when the output resistance stays in the middle, both LEDs are

off.

The binary prototype is shown in Figure 2. The output of the sensor is connected with a fixed resistor serially. With the help of the voltage divider, the output resistance is converted to a voltage signal, which is proportional to the output signal. The voltage signal is fed to a comparator and compared to a threshold voltage, which is created by another voltage divider. A potentiometer is used to create the threshold voltage, so that it is very easy to adjust the threshold. The comparator compares the two inputs and generates a output signal that either turns on or turns off the following LED.

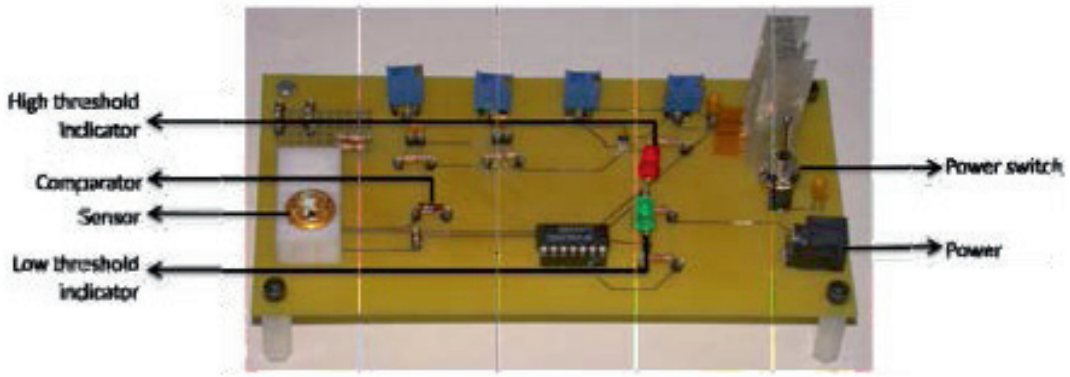

Figure 2: Photograph of designed binary portable device for disease diagnosis.

The sensor is protected from the environment by a chamber. A specially designed channel allows exhaled human breath flow or controlled gas flow to go through the chamber and interact with the sensor. Figure 3 shows a demonstration of the use of the binary prototype. **Ammonia** [15], **and acetone breath analyzer** [9] prototypes respectively have been produced and reported by our research team, based on single breath single-gas detection.

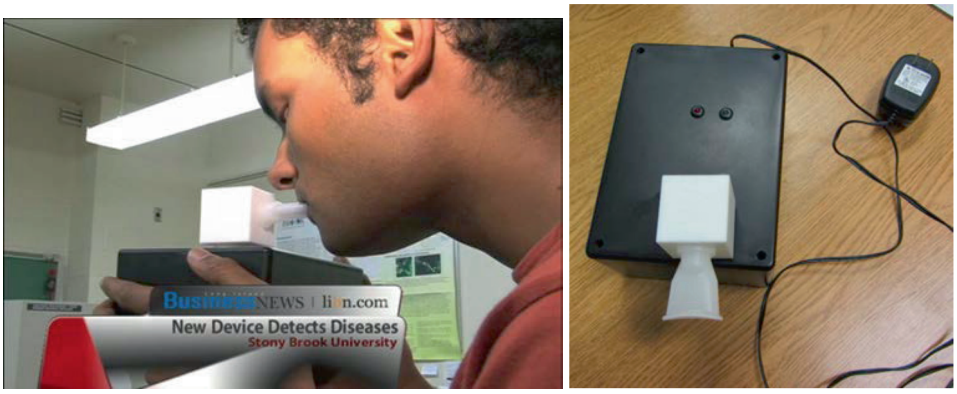

Figure 3: Demonstration of the use of the binary breathalyzer prototype for a TV news segment; photo of the binary breathalyzer produced by our group.

4. Current Status

4.1. Scalable synthesis of Nanowire Sensors

WO_3 nanoparticles were synthesized by flame spray pyrolysis [16] using the desktop nanoparticle synthesizer (FSP) (NP10, Tethis) housed in P. Gouma's lab at SUNY Stony Brook. To prepare the precursor solution, 0.38 M of Tungsten(VI) isopropoxide (99%, All-Chemie) was dissolved in 2-propanol within N_2 filled glove box. This solution was supplied at a rate of 5 ml/ min through the FSP nozzle and dispersed to a find spray to a fine spray with 5 l/min oxygen. The fine spray was ignited by a supporting gas rate (CH_4 :1.5 l/min and O_2 : 3.2 l/min). The synthesized powder was deposited on glass-fiber filter (Whatman).

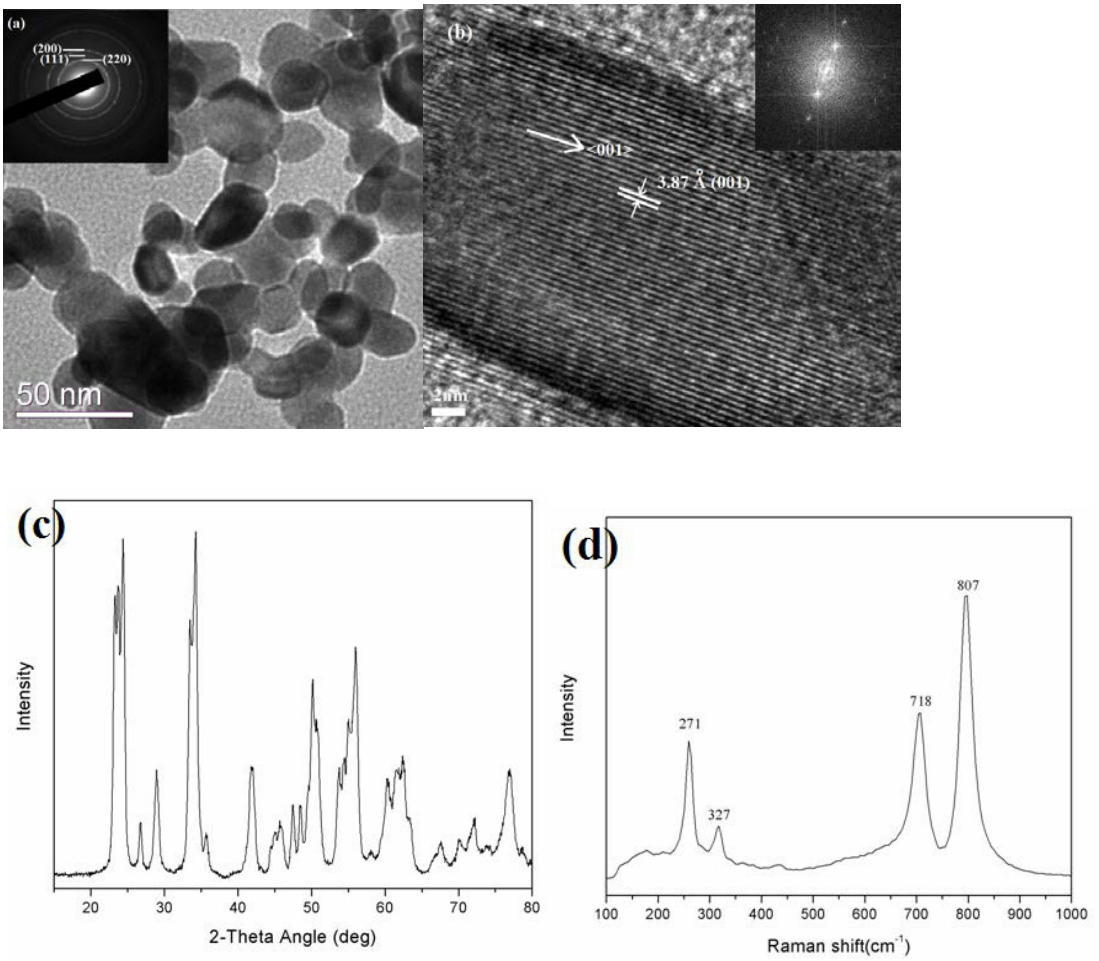

Figure 4 (a) Transmission electron micrograph of the flame synthesized nanoparticles of WO3, with the corresponding SAED pattern; (b) shows the HRTEM of the nanoparticles which have a clean single crystalline surface with the highlighted lattice spacing corresponding to the (001) plane (Inset: corresponding FFT); (c) shows the XRD patterns of WO3 nanocrystals prepared from the rapid solidification process; (d) Shows the corresponding Raman spectra of WO3 nanoparticles. The peaks at 271, 327,718 and 807 cm-1 are typical of ReO3-type WO3 [17].

Figure 4 shows the morphology and structure of the material and confirms the presence of the orthorhombic phase of WO_3 crystals (JCPDS No.: 20-1324). The calculated particle size from the Scherer's formula was 20 nm, consistent with the results of the TEM analysis. Raman spectroscopy results confirm the absence of W-C or W-OH

bond vibrations thus suggesting a monocrystalline material. The peaks at 271, 327,718 and 807 cm^{-1} are typical of ReO_3-type WO_3 [17].

The flame-synthesized nanoparticles were used to prepare sensors. To prepare the sensing film, 0.02g of the as-synthesized WO_3 powder was mixed with ethanol and the solution was ultrasonically stirred for 30 min. 15 drops of the suspension were deposited on a Pt electrode-coated Al_2O_3 substrate. The substrate was dried in air for 1 h then placed in the oven for additional drying at 75°C for 1h. The steps outlined above were repeated 3 times to obtain uniform deposition of WO_3 nanoparticles on the sensing substrate. The sensing substrate with the nanoparticles was then heat-treated to 500°C to stabilize the phase.

The gases used were UHP Nitrogen, UHP Oxygen, and 10 ppm nitric oxide in nitrogen (all of them were obtained from Global Calibration Gases). The flow rates of the gases were controlled in the gas flow bench by a 247-MKS 4-channel readout and 1479 MKS mass flow controllers in the unit of sccm (standard cubic centimeter per minute). The sensor was placed inside in the quartz tube inside of a tube furnace (Lindberg/Blue) that could be heated at a programmable rate. The changes in the electrical resistance of the sensing film were monitored by an Agilent 34401A digital multimeter (Alfa Aesar, 0.25 mm diameter, 99.998%) and were recorded by the software (Agilent Digital Multimeter Connectivity Utility) on the computer. Sensor testing started when the sensor resistance baseline was stable in the background air (8 to 1 nitrogen and oxygen) at working temperature. The films were found to be highly sensitive to low concentrations of Nitric Oxide.

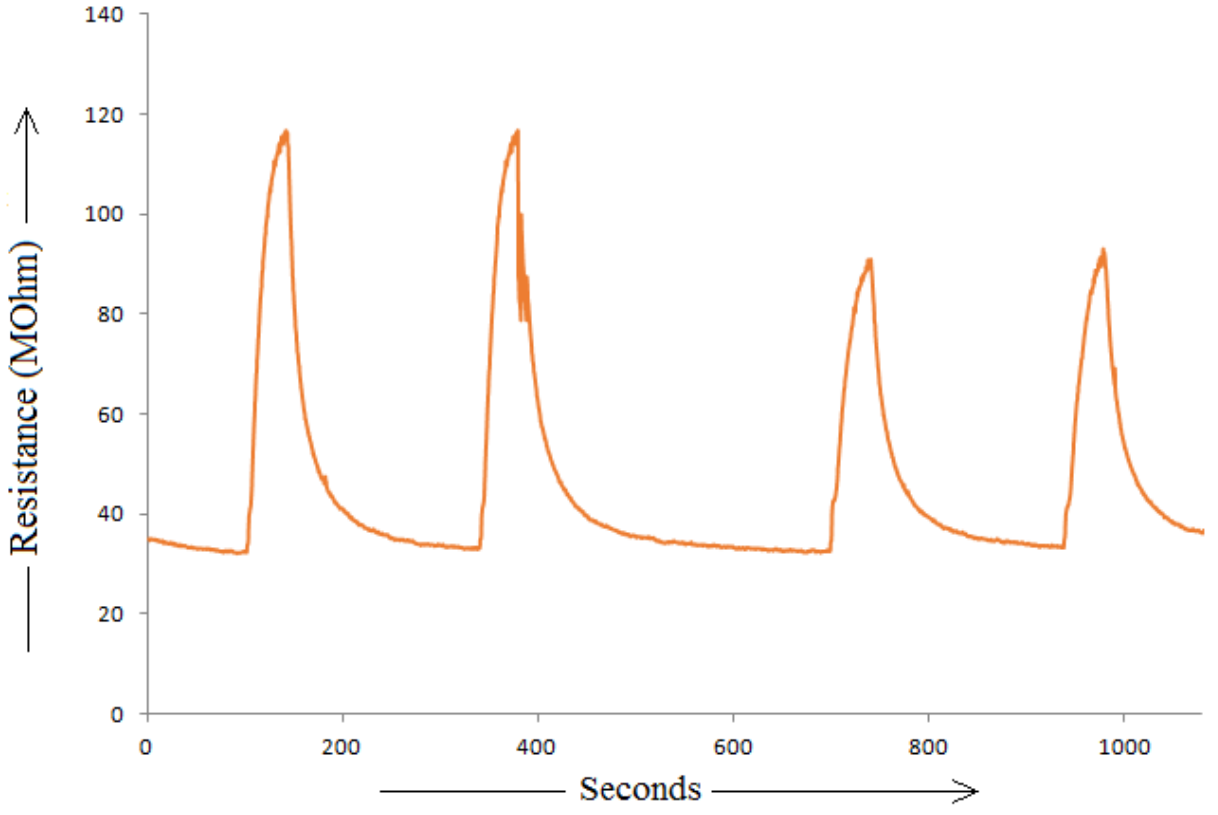

Figure 5: NO sensing test results (resistance vs time) for the flame sprayed WO3 nanoparticles exposed to 500ppb and 300ppb of nitric oxide gas.

Figure 5 shows typical data for the sensitivity of the nanoparticles to nitric oxide at 500 parts per billion (sensitivity

value of ~ 3.8) and at 300 parts per billion (sensitivity value of ~ 3.4). This is the best sensitivity reported for NO detection by pure WO_3 and it is attributed to the uniform structure and morphology of the flame-processed nanoparticles. The working temperature of the sensor was set at 350°C. At temperatures below 350°C the sensor did not recover to its baseline resistance values whereas at temperatures above 400°C the nanoparticles had an unstable baseline.

NO is excreted in human airways and is detectable in exhaled air in significant amounts ranging from 0.2–1 ppm in the upper respiratory tract and 1–30 ppm at the nasal level. The sensor described above may be easily used for the detection of NO in exhaled breath.

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