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Performance of Amperometric Platinized-Nafion Based Gas Phase Sensor for Determining Nitric Oxide (NO) Levels in Exhaled Human Nasal Breath

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Abstract: Nitric oxide (NO) levels in exhaled breath are a non-invasive marker that can be used to diagnose various respiratory diseases and monitor a patient's response to given therapies. A portable and inexpensive device that can enable selective NO concentration measurements in exhaled breath samples is needed. Herein, the performance of an amperometric Pt-Nafion-based gas phase

sensor for detection of NO in exhaled human nasal breath is examined. Enhanced selectivity over carbon monoxide and ammonia is achieved via an in-line zinc oxide-based filter. Exhaled nasal NO levels measured in 21 human samples with the sensor are shown to correlate well with those obtained using a chemiluminescence reference method ($R^2=0.9836$).

Keywords: amperometric gas-phase sensor • nitric oxide • nasal breath • Nafion • zinc oxide

Nitric oxide has been extensively studied as a biomarker for over 25 years. Changes in exhaled oral and nasal NO levels have been associated with numerous respiratory diseases, and successfully used to diagnose asthma and monitor response to anti-inflammatory therapy [1–4]. While exhaled oral NO levels (eNO) are very low, ca. 10–35 ppbv and 5–25 ppbv in healthy adults and children [5], respectively, exhaled nasal NO concentrations (nNO) are typically much higher, in the range of 200–1400 ppbv (parts per billion by volume) [6]. Nitric oxide levels are particularly high in the paranasal sinuses [7]; however, nasal cavities, the middle ear, and nasopharynx areas can also contribute to the nNO output [8]. Decreased levels of nNO have been observed in patients with primary ciliary dyskinesia (PCD) [9], cystic fibrosis (CF) [10], and chronic rhinosinusitis (CRS) [11]. PCD is a rare, autosomal recessive disease that occurs in approximately 1 in 15,000–20,000 individuals [12], whereas ca. 30,000 people with cystic fibrosis live in the U.S. alone [13]. Chronic rhinosinusitis affects 13–17% of the U.S. population [14] and bacteria associated with CRS infections are often difficult to completely eradicate with conventional antibiotic treatment owing formation of biofilm that protects the microorganisms [15]. However, NO is known to be a potent antimicrobial agent in our bodies [16], and can also disperse biofilms, making antibiotics more effective [17]. Thus, detecting lower than normal levels of nNO in patients with CRS could be helpful in developing a suitable treatment plan.

A highly sensitive and selective gas phase chemiluminescence method is the ‘gold standard’ for breath NO measurements. The U.S. FDA cleared a chemiluminescence-based NIOX breath NO test system for clinical use in May 2003 [18]. However, the use of chemiluminescence analyzers have found limited routine use for clinical

applications due to their very high cost, frequent maintenance requirements, and the need to use an ozone generator. Therefore, various electrochemical sensors have been explored for use in exhaled NO measurements, and several devices are currently on the market. In 2014, the National Institute for Health and Care Excellence (NICE) assessed the performance of three of these devices: NObreath from Bedfont Scientific Ltd, as well as the NIOX MINO and NIOX VERO from Aerocrine. The goal of this study was to help the National Health Service (NHS) decide on whether to recommend the use of these products for measuring fractional exhaled NO (FeNO) concentration in asthma patients. These devices allow for fast detection of very low NO levels in the range of 5 to 300 ppbv. However, only the NIOX MINO device is currently approved by the U.S. FDA for FeNO measurements in asthma patients [19]. Moreover, Medisoft has also released a stationary and semiportable device (~10 kg) (the FeNO+ product), that can be used in 3 testing modes: bronchial, alveolar, and nasal, with the nasal one being optional. However, a smaller device that enables NO determination at slightly higher levels, up to 2 ppmv, is still needed for PCD, CF, and CRS diagnosis and therapy.

It has been shown that NO can be detected amperometrically at a noble metal electrode deposited on a solid polymer electrolyte (SPE) membrane like Nafion over a wide concentration range [20–23]. The lower detection

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limit of these sensors is typically 5 ppbv [23,24], which meets the requirements for nNO determinations [25]. The potential at the working electrode is generally kept above 0.95 V vs. Ag/AgCl/3 M NaCl to operate in a diffusion limited region [26]. However, at this high potential, other gaseous species can be electrochemically oxidized at the electrode surface and can contribute to the sensor's overall response. The inability to discriminate between different analytes has proven to be a significant limitation of amperometric SPE-based sensors. Enhancing the selectivity is challenging. Essentially, approaches examined to date rely on the use of a catalysts applied to the electrode surface, optimized electrolyte compositions, and/or the use of complex diffusion barrier membranes and in-line filter systems. There are over 800 volatile organic species and inorganic gases present in the human exhaled breath [27] so the number of potential interfering species is extensive. Therefore, it is important to address at least the most significant known interferences, such as ammonia and carbon monoxide (CO), that can be found in the exhaled breath at concentrations up to 1 ppmv and 10 ppmv, respectively [28].

In our previous work we reported a highly sensitive amperometric Pt-Nafion based gas-phase NO sensor for the characterization of NO generation/release rates from NO-donor biomaterials and devices [24]. An excellent correlation was found for measurements with this sensor vs. chemiluminescence results. However, there were no interfering species present in the tested NO release samples. The sensor has been fully characterized in that prior article, but has never been used in human studies. In this paper, we focus on enhancing Pt-Nafion based sensor's selectivity over NH_3 and CO *via* use of an on-line filtration/scrubber in the gas stream. The applicability of this more selective electrochemical system is verified by testing human nasal breath samples and comparing the results with those obtained using the well accepted chemiluminescence method.

As mentioned above, filtration and scrubbing can be used to remove known interfering species from the gas stream. Acid liquid scrubbers, citric acid granules and a commercially available ammonia scrubber, were all only able to remove ammonia gas produced by injecting an ammonium chloride solution (250 μL , 1 M) into 5 mL of 1 M NaOH and purging that solution with nitrogen into the inlet of the sensor (see Figure 1-S). However, the selectivity over carbon monoxide did not change significantly with such filters (10 ppmv CO was from a commercial tank). When there was no filter or scrubber placed in line, the $\log K_{\text{NO,CO}}$ (log of selectivity coefficient, where $K_{\text{NO,CO}}$ is the ratio of sensor's sensitivity obtained for CO to sensitivity towards NO) for the Pt-Nafion based electrode was -2.16 ± 0.06 and it decreased only to -2.36 ± 0.03 and -2.31 ± 0.08 when using citric acid granules or a commercial ammonia scrubber, respectively. When the incoming gas stream was purged through either sulfuric acid, or hydrochloric acid solutions, the $\log K_{\text{NO,CO}}$ decreased to -2.64 ± 0.15 . However, in this arrangement

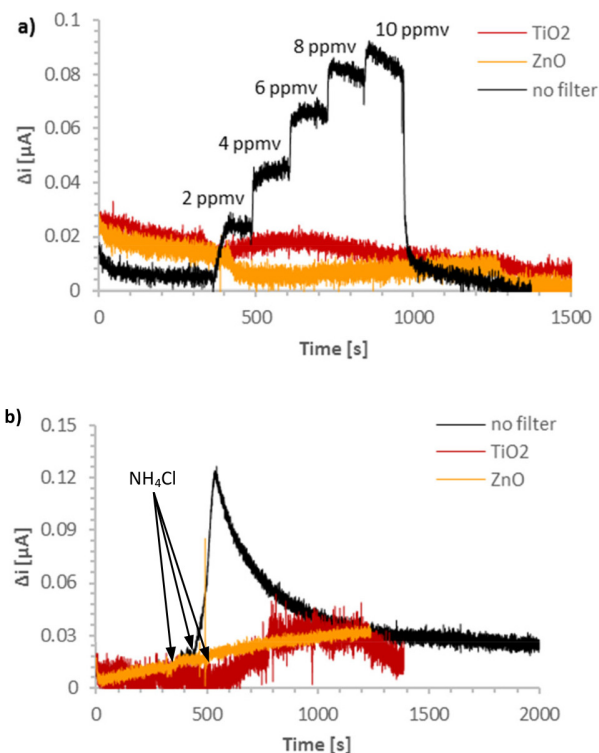


Fig. 1. (a) Pt-Nafion based NO sensor's response to CO standard gas (in N_2 background) in 2 ppmv increments without and with TiO_2 or ZnO filter/scrubber in place at a flow rate of 200 mL/min, and (b) response of NO sensor to ammonia gas produced in situ by ammonium chloride injection into 1 M NaOH solution that is purged at a flow rate of 50 mL/min with nitrogen into sensor with or without TiO_2 or ZnO scrubber in place.

droplets can be created and deposited on the working electrode, which can poison the electrode surface and leads to unstable signals during measurements.

It was previously shown that a proprietary carbon fiber filter (Aerocrine AB, Sweden) can be used to pretreat breath samples before they were analyzed electrochemically [29]. However, in our study, carbon materials, including activated charcoal and carbon fiber cloth, turned out to be too aggressive and removed not only interfering species but also a significant fraction of NO. In contrast, both a titanium (IV) oxide catalyst support and a zinc oxide-based material were found to efficiently remove CO from a gas stream (Figure 1a). The selectivity over CO is increased by around one log unit when the ZnO or TiO_2 filter is present in line ($\log K_{\text{NO,CO}} = -2.16 \pm 0.06$ in absence, and $\log K_{\text{NO,CO}} < -3$ with the either filter, respectively, $n = 3$).

Moreover, as can be seen in Figure 1b, both filters/scrubber also effectively remove ammonia from the gas stream. Nevertheless, the ZnO filter has slightly better performance than TiO_2 filter. Further, no significant decrease in sensitivity towards NO is observed with the either of these filters/scrubbers in place (1268 pA/ppbv for no filter vs. 1228 pA/ppbv and $\text{LOD} = 3.8 \pm 0.8$ ppbv based

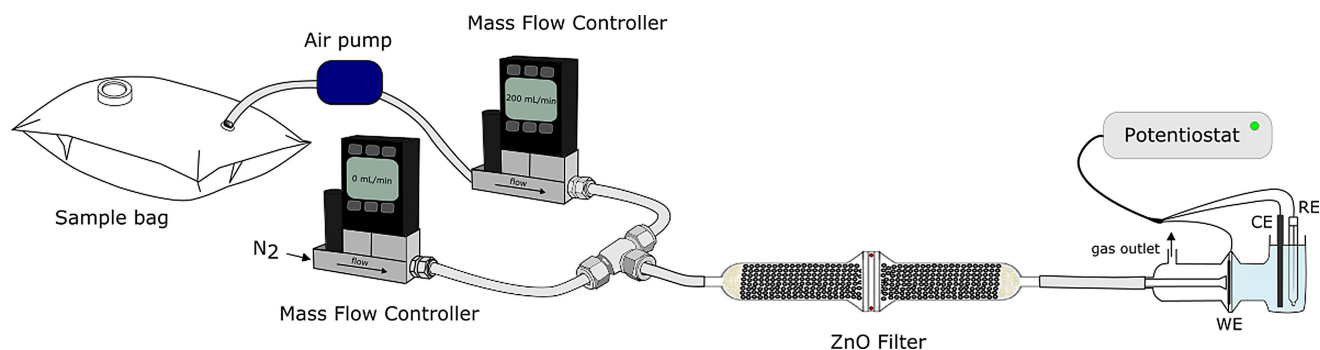


Fig. 2. Sensing configuration employed for nitric oxide level determination in nasal breath samples.

on $3 \times \text{SD}$ obtained for blank/slope, $n=5$, with ZnO filter). For these reasons, the ZnO HiFUEL[®] A310 filter was used for subsequent nasal breath sample measurements with the NO gas phase sensor.

The schematic diagram of the electrochemical sensor system with the ZnO filter in place is shown in Figure 2. The nasal breath samples were collected in a non-nitric

oxide permeable bag. Each nasal breath sample was analyzed 3 times with electrochemical sensor and the average value was calculated.

Figure 3 shows the Pt-Nafion based sensor's current response to a nasal breath sample evacuated at a flow rate of 200 mL/min without and then with the filter present in line. With no filter, the current response is ca. 4 times greater than with the ZnO scrubber in place. Furthermore, the corresponding NO level obtained in the system with a scrubber was found to be in a good agreement with the one measured using the reference chemiluminescence method (35.4 ± 0.5 ppbv and 30.1 ppbv measured using amperometric and chemiluminescence methods, respectively). This suggests that the ZnO material efficiently removes the majority of interfering species present in the nasal breath sample that can be oxidized at the Pt working electrode of the gas phase sensor. Moreover, as can be seen in Figure 4, the amperometric Pt-Nafion based sensor's alternate response to the sample gas is quite stable and reproducible. Hence, no electrode surface contamination occurs during the measurements and the same sensor can be used to detect NO in many nasal breath samples with no need for electrode regeneration.

The filter/scrubber-based sensor system was then used to determine NO levels in 21 samples of nasal NO obtained from 9 human subjects on different days. The correlation and agreement between the data obtained using the amperometric sensor and the chemiluminescence method for nasal breath samples were verified by linear regression and Bland Altman analysis [30,31] (Figure 5). A very good linear relationship was obtained within the range of 32.2 to 190.8 ppbv of NO (based on chemiluminescence) resulting in $R^2=0.9836$. The slope of the linear regression curve was 1.187 ± 0.035 indicating that amperometric sensor reads slightly higher values than a chemiluminescence analyzer at the higher concentrations. Moreover, the differences between the two measurements presented *via* Bland Altman plot shows a relatively low bias of -11.2 ppbv and an agreement range from -35.7 ppbv to 11.9 ppbv. A moderate negative trend of differences, proportional to the magnitude of the measurement was also found (Figure 5b). This negative bias is mostly caused by the sample measurements over

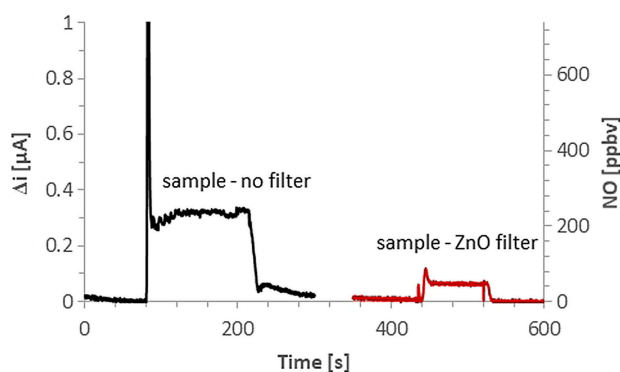


Fig. 3. Amperometric Pt-Nafion sensor's response to exhaled human nasal breath sample without and with ZnO based filter present in line obtained by applying 1 V vs. $\text{Ag}/\text{AgCl}_{\text{sat'd}}$ and using 0.5 M H_2SO_4 as internal electrolyte solution.

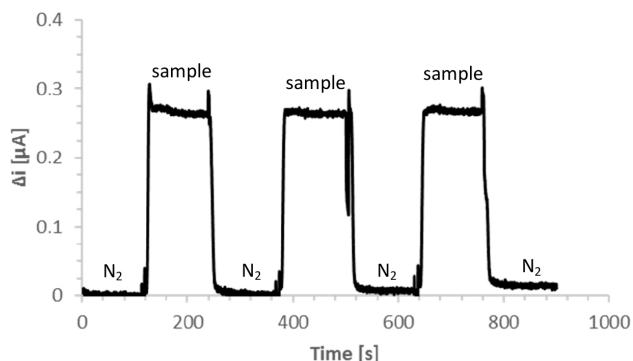


Fig. 4. Amperometric Pt-Nafion sensor's alternate response to nasal breath sample and nitrogen at flow rate of 200 mL/min obtained by applying 1 V vs. $\text{Ag}/\text{AgCl}_{\text{sat'd}}$ and using 0.5 M H_2SO_4 as internal electrolyte solution.

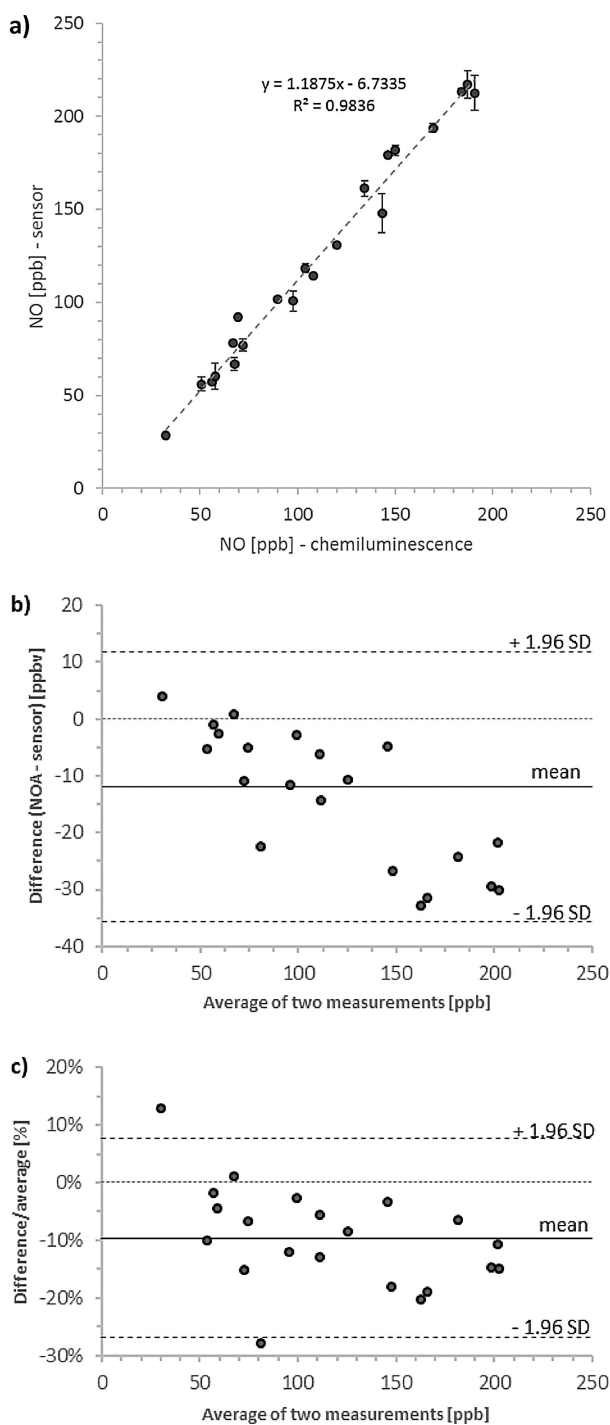


Fig. 5. Comparison of nasal nitric oxide values obtained using amperometric sensor and chemiluminescence method: (a) linear regression analysis; (b) Bland and Altman plot where differences are presented as units (ppbv) and (c) Bland and Altman plot where differences are presented as percentage.

140 ppbv. When the differences are plotted as percentage of the concentration (Figure 5c) the bias is -9.6% and the agreement range ($1.96SD$) is $\pm 8.8\%$. This bias is rather constant for all the measurements.

It should be noted that the NO levels measured in this study are considerably lower compared to the values that can be found in the literature for nasal breath samples, i. e. 200–1400 ppbv [6]. This is directly related to the sample collection method. In the process of exhalation through the nose, a constant transnasal flow rate produces a washout phase followed by the establishment of a steady NO plateau [25]. Therefore, since the gaseous samples were collected in a bag prior to the analysis, the measured NO level reflected the average concentration of those two phases. In addition, nasal NO concentration is inversely related to the transnasal airflow rate [32] and the subjects in this study were likely exhaling at flow rates that were much higher than in prior reports. Indeed, different flow rates can result in different aerodynamic profiles, leading to changes in the physics of airflow and different path flows through the nasal cavities which will affect the nasal NO output [33,34]. In general, it takes less than 30 s to fill the 2 L sample collection bag; therefore, NO can be diluted to the greater extent and lower values were determined. Nevertheless, in a separate experiment, the Pt-Nafion based sensor was able to reliably measure NO concentrations even up to 4 ppmv (Figure 3-S), thus making it applicable for nNO determination when controlled gas flows are much lower and nNO levels are much higher than in samples analyzed in this study.

In summary, it has been shown that an amperometric Pt-Nafion based gas phase sensor is capable of determining NO levels in exhaled human nasal breath. Enhanced selectivity over two main interfering species, namely ammonia and carbon monoxide, was achieved by on-line sample pretreatment using zinc oxide-based filter. The sensitivity toward NO remained unchanged after filter introduction suggesting that there was no interaction between the zinc oxide-based material and NO. A detection limit of 3.8 ± 0.8 ppbv ($n=5$) was achieved which meets the requirements for nNO determinations. The results obtained using the amperometric gas phase sensor with an in-line filter for determining nNO levels in human nasal samples were in good agreement with those measured with the reference chemiluminescence method. It is envisioned that this new sensor approach could be useful in developing a low-cost NO measurement system to help diagnose and monitor the treatment of patients with primary ciliary dyskinesia, cystic fibrosis and chronic rhinosinusitis.

Experimental

Nitric acid (65%), sulfuric acid (95–98%), hydrochloric acid (37%), phosphoric acid (85%), citric acid (99%), sodium hydroxide (99.998%), sodium borohydride (99.99%), ammonium chloride (99.5%), and tetraamineplatinum(II) chloride hydrate (98%) were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Zinc oxide based sulfur removal material, HiFUEL®A310, spheres of 2–5 mm dia. was obtained from Perma Pure LCC (Lakewood, NJ). Ammonia scrubbing media and

titanium (IV) oxide catalyst support were purchased from Alfa Aesar (Ward Hill, MA). All gases used for the calibration of amperometric sensors were purchased from Cryogenic Gases (Detroit, MI). Ultrapure water from a MiliporeMili-Q system (Milipore, Bedford, MA) was used to prepare all the aqueous solutions. Sensor cell assembly and filter housing parts were prepared by Glass-blowing Services, Department of Chemistry, University of Michigan (Ann Arbor, MI). All herein reported potentials are versus Ag/AgCl (saturated KCl).

A Pt-Nafion working electrode was fabricated according to the previously described method [20,23] which was also applied in our earlier work [24] with minor modifications. Briefly, a 1.6 cm dia. circles were cut out from Nafion 117 sheet (DuPont, Wilmington, DE) and cleaned of impurities by boiling in 3 M nitric acid for 1 h and then in deionized water for 1 h. Platinum was deposited onto/into the polymer electrolyte membrane using the impregnation-reduction method [35]. Nafion membrane was placed between two glass cells with 0.94 cm dia. openings (apparent geometric area of 0.88 cm²) with one side exposed to 2 mM Pt(NH₃)₄ Cl₂ solution and incubated for 20 h at 37 °C. Then, 50 mM NaBH₄ in 1 M NaOH was placed on the same side of the glass cell. The chemical reduction of the residual Pt(NH₃)₄ Cl₂ was allowed to proceed for 1 h at 37 °C. Afterwards, the Pt-Nafion membrane was boiled in deionized water for 1 h to remove any remaining Pt complex and reducing agents. The membrane was then mounted in a glass sensor assembly with metallic side of the electrode facing the gas phase. A single junction Ag/AgCl_{sat'd} reference electrode and Pt wire auxiliary electrode were placed in the liquid chamber filled with 0.5 M H₂SO₄ internal electrolyte solution. A 10 mm × 2 mm piece of 50 μm thick Au foil was used as the working electrode lead, and secured between the SPE membrane electrode and the gas inlet/outlet section of the sensor. The glass cell containing impregnation/ reduction solution had a small channel next to the opening, and a small platinum edging was created to facilitate the current collector connection. After assembling the sensor, the electrode potentials were scanned between -0.3 and 1.5 V for 20 cycles at a scan rate of 20 mV/s under a nitrogen atmosphere at a flow rate of 200 mL/min to clean the electrode/electrolyte interface. To measure nitric oxide levels 1 V vs. Ag/AgCl_{sat'ed} was applied to the working electrode and a sensor's output current was recorded using a CHI800 potentiostat (CH Instruments, Austin, TX). The potential applied to the working electrode provides the highest sensitivity of the sensor and ensures the operation in the diffusion/mass transport controlled region [24]. The final sensor assembly can be seen in Figure 2-S in Supplemental File, and in the far-right portion of Figure 2 above. MC-200SCCM mass flow controllers (Alicat Scientific, Tucson, AZ) were used to deliver calibration gas and gaseous sample to the gas phase sensor at a constant flow rate.

For filtration through solid particles, a gas column with inlet and outlet was packed and placed immediately upstream from the sensor's gas inlet. Activated charcoal, citric acid granules, ammonia scrubbing media, titanium

(IV) oxide catalyst support, and zinc oxide based sulfur removal material were all prepared and tested in this fashion. Filtration through a carbon fiber cloth (Charcoal House LLC, Crawford, NE) was also examined by placing the filter material between two glass cells with inlet and outlet and at a location before the sensor's gas inlet. The edges of the thin filter were sealed off by o-rings. For acid liquid scrubbing, a glass cell was filled with 3 mL of scrubbing solution (0.5 M H₂SO₄, 0.5 M HCl, 0.5 M H₃PO₄), and purged with nitrogen through fritted glass (medium coarse) prior to experiments.

Nasal breath samples were collected in 2 L non-diffusing gas collection bag (5-layer laminate with foil center and polyethylene inner layer) equipped with a 3-way stopcock using nasal mask (Hans Rudolph, Inc., Shawee, KS) according to a University of Michigan IRB approved protocol. Hytrel tubing (Vacumed, Ventura, CA) was used to connect the nasal mask to the gas collection bag, and the mask contained a single-patient particle, bacterial, and viral filter (Aerocrine, Sweden). Volunteers were asked to inhale orally and then immediately expire through nose at as a constant flow rate as possible. Samples were evacuated from the bag using a micro air pump TCS D3K (Servoflo Corp., Lexington, MA) and analyzed electrochemically at a constant flow rate of 200 mL/min. Nitric oxide levels in the nasal samples were also measured from the sample bag (before the sensor measurements) using a Sievers chemiluminescence Nitric Oxide Analyzer[®] (NOA) 280i (GE Analytical, Boulder, CO).

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