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Highly Sensitive Amperometric Pt-Nafion Gas Phase Nitric Oxide Sensor: Performance and Application in Characterizing Nitric Oxide-Releasing Biomaterials

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Abstract

A highly sensitive amperometric gas-phase nitric oxide (NO) sensor based on a Pt working electrode chemically deposited on a Nafion film is described. The Pt electrode is chemically deposited on a Nafion 117 membrane by impregnating the film with $\text{Pt}(\text{NH}_3)_4^{2+}$ ions, which are then exposed to NaBH_4 to precipitate conductive Pt metal. The sensor was characterized with a mass-flow controlled 1 ppm NO standard gas and has an electrochemical surface area of $34 \pm 9 \text{ cm}^2$, low limits of detection ($4.3 \pm 1.1 \text{ ppb}$) and a fast response time ($<5\text{s}$) toward changes in gas phase NO levels. Good correlation was found for measurements with the new amperometric Pt-Nafion sensor vs. chemiluminescence results for detecting the rates of NO released from CarboSil 2080A polymer films doped with *S*-nitroso-*N*-acetylpenicillamine ($R = 0.999$, $m = 0.999$, $n = 6$) and for electrochemical reduction of nitrite to NO ($R = 0.999$, $m = 0.938$, $n = 3$) mediated by a copper(II)-tri(2-pyridylmethyl)amine complex.

Graphical Abstract

Keywords

nitric oxide; *S*-nitrosothiol; amperometric gas-phase sensor; Nafion; electrochemical nitric oxide generation

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1. INTRODUCTION

Nitric oxide (NO) has been studied extensively since its discovery as the endothelial-derived relaxing factor in 1987 [1–3] and has since been shown to play critical roles in a number of other physiological functions such as inhibition of platelet activation/adhesion [4, 5], neurotransmission [6, 7], and as a potent antimicrobial agent [8]. NO is produced endogenously during the oxidation of L-arginine to L-citrulline via heme-based enzymes known as NO synthases [4, 5]. Local changes in NO concentration *in vivo* are often important indicators of physiological and biochemical phenomena. For example, exhaled NO concentrations (either oral or nasal) may be useful in diagnosing asthma and other respiratory diseases such as chronic rhinosinusitis (CRS) [9, 10], that affects 13–14% of Americans [11, 12].

Due to the attractive antithrombotic and antimicrobial properties of NO, investigations of synthetic NO-donor molecules, such as *S*-nitrosothiols (RSNO) [13, 14] and diazeniumdiolates [14], incorporated into medical-grade polymers (e.g., silicone rubber, polyurethanes, and block co- or tri-polymers of these and other polymers [15]), have been undertaken in order to improve the biocompatibility of catheters and other implantable medical devices. Currently, the rates of NO release from these materials are usually characterized by ozone-based chemiluminescence [16]. Similar chemiluminescence measurements are also the gold standard for measurement of NO in exhaled oral and nasal breath samples [17–19]. While chemiluminescence is highly sensitive (with a purported limit of detection of 0.5 ppb [20]) and high selectivity for NO, it remains costly as a workhorse analytical-research technique and is relatively impractical as a near-patient diagnostic tool due to its complexity and size.

Amperometric NO sensing is inexpensive, compact, and quite facile to integrate into devices, making it an attractive alternative to chemiluminescence. Clark-type [21–24] and solid microsensor [25, 26] varieties of amperometric NO sensors have been developed over the last 25 years for detection of NO dissolved in aqueous phases. For gas phase NO measurements, semiconductor [27–29] and solid-polymer electrolyte (SPE)-based sensors [30–34] have been reported. While conductivity-based sensors have good stability, they often require high operating temperatures and fall short of the requisite response times and limits of detection for detecting NO from exhaled breath (10 – 500 ppb) and from NO-donor doped materials (5 ppb – 100 ppm).

SPE-based sensors utilize ion-exchange membranes such as Nafion 117 as both a high surface area scaffold for the deposition of a metal working electrode and as an ion conductive pathway for the electrochemical cell. SPE-based amperometric sensors have been developed for a wide range of gaseous species that include volatile organic compounds such as ethylene [35] and small inorganic molecules such as SO₂, CO, NO and H₂S [32–34]. Prior SPE-based NO sensors reported in the literature [30, 31, 36] have been based on platinized Nafion and report limits of detection of <10 ppb, with fast response times and large dynamic linear ranges. Despite the promising initial characterizations of amperometric Pt-Nafion NO sensors, no studies have been conducted to demonstrate their potential biomedical/biomaterials-research applications or to establish a correlation between data

obtained using Pt-Nafion amperometric NO sensors versus that of the gold standard for gas phase NO detection, chemiluminescence.

In this work, an optimized gas-phase amperometric Pt-Nafion SPE type NO sensor is described and applied for determining the rates of NO released from RSNO-doped polymer films as well as NO generated electrochemically via copper(II)-tri(2-pyridylmethyl)amine (CuTPMA) mediated reduction of inorganic nitrite within NaNO₂ solutions [37]. The RSNO and polymer of choice in this work are *S*-nitroso-*N*-acetylpenicillamine (SNAP) and CarboSil2080A, respectively. Replicate samples of the same NO release materials are also analyzed for NO release rates via chemiluminescence measurements and the results are compared to those obtained via the new amperometric sensing approach. While selectivity concerns are minimal for NO generated from NO-donor materials, preliminary selectivity experiments are also described for the SPE NO sensor with respect to carbon monoxide (CO), a known potential interfering species in exhaled breath.

2. EXPERIMENTAL

All chemicals used were commercially obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise specified and were reagent grade without further purification or modification. All gases used for the calibration of the Pt-Nafion sensors were obtained from Cryogenic Gases (Detroit, MI). Aqueous solutions were prepared using water (18.2 MΩ-cm) purified through a Milli-Q system (Millipore, Bedford, MA). Sensor cell assembly and sample cells were prepared by Glass-blowing Services, Department of Chemistry, University of Michigan (Ann Arbor, MI). All potentials reported herein are versus Ag/AgCl (saturated KCl), which is +0.197 V vs. SHE.

2.1 Sensor Fabrication

The general design and fabrication of the Pt-Nafion working electrode has been described in previous reports [30, 31, 35, 38, 39], with minor changes described in this section. Briefly, Nafion 117 (DuPont, Wilmington, DE) was cut into 1 cm dia. circles and cleaned of impurities by boiling in 3 M nitric acid for 1 h followed by boiling in deionized water for 1 h. The membranes were then incubated in 20 mM Pt(NH₃)₄Cl₂ at 37 °C for 6 h before mounting between two glass cells with 0.94 cm dia. openings (0.88 cm² exposed geometric area) with one side of the ionomer membrane exposed to 50 mM NaBH₄ in 1 M NaOH. Chemical reduction was allowed to proceed for 90 min. The Pt-Nafion membrane was then boiled in DI water for 1 h to remove any remaining Pt complex and reducing agent. The working electrode lead was a 10 mm x 2 mm piece of 50 μm thick Au foil secured between the SPE membrane electrode and the gas inlet/outlet section of the sensor (made out of glass). A single junction Ag/AgCl (saturated KCl) reference electrode and bare Pt auxiliary electrode were used along with a 0.5 M H₂SO₄ internal electrolyte in the final sensor assembly (see Figure 1). A CHI800B potentiostat (CH Instruments, Austin, TX) was used to polarize the working electrode and record sensor output currents. Note that in all measurements, the metallic side of the membrane electrode faces the gas phase. 2x MC-200SCCM mass flow controllers (Alicat Scientific, Tucson, AZ) operated in tandem via a BB9-USB signal distribution box (Alicat Scientific, Tucson, AZ) and FlowVision SC software (Alicat Scientific, Tucson, AZ) were used for mixing of calibration gas (1 ppm NO

in N₂ balance and 10 ppm CO in N₂ balance) and delivery of nitrogen into sample cells (with RSNO doped films or electrochemical NO generation from nitrite) to advance the NO released from the samples to the gas phase sensor.

2.2 NO-Release Film Preparation and Characterization

The general concept for preparation of the SNAP-doped CarboSil2080A (DSM Biomedical Inc., Berkley, CA) films has been described in a previous work [15] although in that earlier work, the polymer of choice was E2As, not CarboSil2080A. Further, unlike reported in [15], no silicone rubber topcoat was utilized in this work. Additionally, SNAP was synthesized from *N*-acetyl-DL-penicillamine (NAP) via a high-yield/high-purity one-step synthesis [15], which is facile and more economical than purchasing SNAP commercially. To prepare the NO release polymer films, 180 mg of CarboSil2080A was dissolved in 3 mL of tetrahydrofuran by gently agitating the mixture for 1 h. Then, 20 mg of SNAP was dissolved in the CarboSil2080A solution and the mixture was poured into a Teflon mold (1 inch dia., 0.25 inch depth). The film was allowed to cure overnight before cutting into 8 mm dia. circles and dried under vacuum for 24 h. The NO-release rate was evaluated via chemiluminescence (Sievers 280i Nitric Oxide Analyzer) and amperometric Pt-Nafion sensing using an arrangement as shown in Figure 2. NO-release from the SNAP-doped polymeric films was modulated via light (S-NO bond is photolytically cleaved at 590 nm [15]) using a broad visible-spectrum fiber-optic ring (DCR II, Schott-Fostec LLC) as the source. The light source has arbitrary light intensity settings that are marked from 0–100; settings of 100, 85, 75, 70, and 65 were used in the modulation of NO release from the SNAP/CarboSil2080A films.

2.3 Electrochemically Modulated NO Release Characterization

In a separate work from our group [37], a novel method to electrochemically modulated NO release was developed and characterized using chemiluminescence. Sodium nitrite in the presence of a copper(II)-complex (CuTPMA), serving as an electron transfer mediator, can be electrochemically reduced to NO at various rates depending on applied potential to a Pt, Pt-Ir alloy, Au or stainless steel working electrode. This technique has the potential to be employed in the development of NO sources for NO inhalation therapy [40, 41] as well as intravascular catheters with a separate lumen for electrochemical generation of NO in order to enhance biocompatibility and reduce risk of catheter associated infections [34]. In this work, the Pt-Nafion gas phase NO sensor was utilized to characterize the rate of NO produced from a bulk solution via the CuTPMA-mediated sodium nitrite electro-reduction reaction and the results were compared to the rates determined under the exact same conditions using chemiluminescence detection of the liberated NO.

The NO-generating solution was composed of 1 mM CuTPMA, 0.3 M NaNO₂, 0.3 M NaCl, and 0.5 M HEPES (buffered to pH 7.2). A CHI1206B potentiostat (CH Instruments, Austin, TX) was employed to modulate the NO generation by applying a constant potential vs. Ag/AgCl (single junction saturated KCl) across a range of potentials. The working electrode was a 6 cm long, 127 μ m dia. Teflon-coated Pt-Ir alloy wire (A-M Systems, Sequim, WA) with an exposed length of 2 cm ($\sim$ 0.08 cm² exposed surface area). The counter electrode was a 10 cm long, 127 μ m dia. Teflon-coated Pt-Ir alloy wire (A-M Systems, Sequim, WA)

with an exposed length of 4 cm ($\sim 0.16 \text{ cm}^2$ exposed surface area), which was coiled around the unexposed section of the working electrode to reduce the space occupied in the sample cell. The experimental configuration for these electrochemical NO generation experiments are shown in Figure 3.

3. RESULTS & DISCUSSION

3.1 Sensor Characterization

Pt-Nafion membranes were characterized by cyclic voltammetry (see Figure S1, Supporting Info) to confirm Pt character and to estimate electrochemical surface area via hydrogen adsorption [36, 42]. An electrochemical surface area of $34 \pm 9 \text{ cm}^2$ was determined for $n = 3$ electrodes prepared as described in the Experimental Section. Amperometric NO sensing performance was evaluated via calibration using a 1 ppm NO standard gas (commercial cylinder of NO) and diluting with nitrogen using mass flow controllers to achieve total flow rates of 50 mL min^{-1} over the surface of the sensor (Figure 4). The response time is quite rapid ($< 5 \text{ s}$) and the sensitivity is favorable ($880 \pm 60 \text{ pA ppb}^{-1}$), with limits of detection (LOD) of $4.3 \pm 1.1 \text{ ppb}$ based on S/N ratio of 3. The sensor response is also highly reversible and reproducible. The optimal operating potential was determined to be $+1.0 \text{ V}$ based on sensitivity studies (see Figure S1, Supporting Info). Previous studies [4, 37] on the effect of flow rate suggests that flow rates beyond 100 mL min^{-1} provide diminishing returns with regard to sensitivity (reaction rate is no longer mass transport limited), which we have confirmed for our sensors as well (see Figure S2, Supporting Info). However, a flow rate of 50 mL min^{-1} was utilized throughout this study because it closely matched the bubbling rate of the chemiluminescence system and does not sacrifice limit of detection or response time to a significant degree. Furthermore, from a practical standpoint, lower flow rates conserve calibration standard gas and reduce splashing, which can contaminate the system plumbing. SEM was also performed to visualize/characterize the surface of the conductive Pt working electrode (see Figures S4–S7, Supporting Info) and these images indicate that the surface has a considerable number of cracks and pores to provide ionic conductivity into the underlying ionomer membrane.

While concern over interfering species is minimal in connection with using the Pt-Nafion sensor to quantitate NO release rates from NO releasing materials, future concerns regarding the sensor's utility in determining NO levels in exhaled breath requires cross-sensitivity evaluation versus carbon monoxide (CO), which is present in exhaled breath at low ppm concentrations [43]. Preliminary findings suggest relatively poor native selectivity for NO vs. CO, which is in disagreement with a previous report [36]; however, the selectivity, expressed as $\log K_{\text{NO,CO}}$ where $K_{\text{NO,CO}}$ is the ratio of CO sensitivity to NO sensitivity, can easily be improved by conditioning the sensor in the presence of CO (Figure 5).

Conditioning involves applying the operating potential to the sensor while under a stream of CO gas (at 10 ppm in N_2). By performing this conditioning for 2 h at a flow rate of 50 mL min^{-1} , selectivity is improved significantly to a $\log K_{\text{NO,CO}}$ value of -2.22 , which indicates a CO sensitivity that is $< 0.6\%$ of the NO sensitivity. The effectiveness of this treatment likely stems from the passivation or blocking of the electrochemically-active sites on the Pt-Nafion electrode towards CO oxidation by the oxidation products of CO (namely, adsorbed CO_2 or

CO₂ adsorption products with co-adsorbed species such as H_{ads} to form COOH_{ads} [44, 45]). Because CO oxidation is generally agreed to follow a Langmuir-Hinshelwood reaction mechanism [44, 46], impedance to its adsorption to the working electrode surface will impede its oxidation. This hypothesis requires further investigation to fully confirm.

Anionic exchange membranes (AEM) may also be used to fabricate SPE-based NO sensors for use with alkaline internal solution, which would promote oxide formation and potentially improve CO selectivity even further [24, 36]. However, AEM commercial availability is limited, and most available products are fabric-reinforced, which is not acceptable for the chemical deposition of metal electrodes. One strategy for promoting oxide formation is to use a higher potential, such as 1.2 V, during the polarization of the sensor before bringing the sensor back to 1.0 V. This strategy, however, greatly reduced the NO sensitivity in our experiments while offering no notable decrease in CO sensitivity. This may be due to the intended oxides blocking the “triple” interfacial sites where the Nafion, Pt, and NO phases meet and where the NO undergoes electrochemical oxidation [34]. Further investigation into this phenomenon is required to gain a clearer understanding of the inability of oxide coatings to enhance NO to CO selectivity with this specific type of sensor.

3.2 Determination of NO Released from SNAP/Carbosil2080A Films

When using the Pt-Nafion gas phase NO sensor to quantitate the photolytic release of NO from SNAP-doped CarboSil polymeric films, the amperometric response of the SPE NO sensor (Figure 6a) closely matches that of chemiluminescence, the reference method for the determination of NO release rates (Figure 6b). This can be attributed to the similarly fast response times (~1 s and < 5 s for chemiluminescence and the SPE sensor, respectively) and low limits of detection as well as the near-identical sampling methods. When NO release rates from identical films are compared between measurements by both methods, not only was strong correlation found ($R = 0.999$), but the results were close to parity ($m = 0.999$) (see Figure S8, Supporting Info). Both methods are calibrated to measure in ppb; however, due to the presence of nitrogen “sweep” gas, the stream in both chemiluminescence and the amperometric Pt-Nafion sensor systems are diluted to different degrees (the Sievers chemiluminescence analyzer system draws a vacuum and operates at a different flow rate that is not optimal for the Pt-Nafion sensor). This makes it difficult to accurately compare ppb measurements from one system to another to report NO release rates. Therefore, in this work, NO-release is reported in absolute pmol s^{-1} , which is independent of sample stream dilution effects. See the Supporting Information file (Section S.4) for a brief explanation of how pmol s^{-1} is derived from ppb gas concentration measurements.

3.3 Determination of Electrochemically Modulated NO Release from CuTPMA/NaNO₂ Solutions

To employ the Pt-Nafion NO sensor to monitor NO release from solutions where NO is generated by electrochemical reduction through CuTPMA mediated electron transfer, the solution phase is purged with a constant flow of nitrogen (at 50 mL min^{-1}) and this gas phase is then flowed into the amperometric sensor (or into the chemiluminescence analyzer to obtain correlation data). The amperometric response of the Pt-Nafion NO sensor (Figure 7a) shows a similar profile compared to that of chemiluminescence measurements (Figure

7b) when the applied voltage to the Pt-Ir working electrode is varied, although the noise level was significantly lower for the new electrochemical sensor method. This may be due to the larger diameter tubing used in plumbing of the Pt-Nafion system compared to that of the chemiluminescence system. Larger diameter tubing may promote mixing/homogeneity of gases when running liquid samples since the bubbling of the solution produces tight bursts of NO that manifest as concentration spikes. Another explanation is that aerosol droplets are formed during aqueous sample purging, which are carried into the reaction cell and scatter the photons detected at the photomultiplier tube of the NOA instrument but have no effect on the electrochemical sensor measurements. The NO-released measured by both methods showed very good correlation ($R = 0.999$) and overall agreement in value ($m = 0.938$) (see Figure S9, Supporting Info). As expected, as the voltage applied is made more cathodic, the rate of generation of Cu(I)TMPA at the electrode surface increases, and this increases the rate of NO generation from inorganic nitrite ions in the solution adjacent to the working electrode surface [37].

4. CONCLUSION

An improved amperometric Pt-Nafion sensor has been demonstrated as a viable and competitive alternative to chemiluminescence for the characterization of NO generation/release rates from NO-donor biomaterials and devices. The gas phase sensor is highly sensitive (880 ± 60 pA ppb⁻¹) with low limits of detection (4.3 ± 1.1 ppb) and a fast response time (<5s). These amperometric Pt-Nafion SPE sensors generate results in close agreement with chemiluminescence in two distinctly different biomaterials-research applications. Selectivity remains a major challenge for use of the amperometric NO gas sensors for diagnostic purposes. However, with proper conditioning and/or use of optimized applied potentials, we have demonstrated that selectivity over CO can be greatly enhanced. While further selectivity studies are needed before applying the sensor configuration described here for measuring NO in exhaled breath (especially enhancing the selectivity over ammonia using a coating over the sensor and/or a trap to remove ammonia), it is certainly possible to use this optimized SPE-based NO sensor at this stage of development for monitoring NO in the gas streams used for NO inhalation therapy within hospital settings (INO-therapy) [40, 41]. With amperometric gas-phase NO sensors of the type described here, it may also be possible to combine electrochemical NO generation [37] and electrochemical NO detection within a new integrated INO system/device that does not require expensive and unstable tanks of compressed NO gas. Research in this direction is currently underway in this laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Ignarro L, Buga G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *PNAS*. 1987; 84:9265. [PubMed: 2827174]
2. Palmer R, Ferrige A, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 1987; 327:524. [PubMed: 3495737]
3. Pueyo M, Darblade B, Rami J. Endothelium-derived Nitric Oxide and Vascular Physiology. *Cell Mol Life Sci*. 1999; 55:1078. [PubMed: 10442089]
4. Radomski MW, Palmer RM, Moncada S. An L-arginine/nitric oxide pathway present in human platelets regulates aggregation. *PNAS*. 1990; 87:5193. [PubMed: 1695013]
5. Moncada S, Higgs E. The L-arginine Nitric Oxide Pathway. *N Engl J Med*. 1993; 329:2002. [PubMed: 7504210]
6. Bredt DS, Snyder SH. Nitric Oxide: A Novel Neuronal Messenger. *Neuron*. 1992; 8:3. [PubMed: 1370373]
7. Vincent SR. Nitric Oxide: A Radical Neurotransmitter in the Central Nervous System. *Prog Neurobiol*. 1994; 42:129. [PubMed: 7480785]
8. Nathan CF, Hibbs JB. Role of nitric oxide synthesis in macrophage antimicrobial activity. *Curr Opin Immunol*. 1991; 3:65. [PubMed: 1711326]
9. Naraghi M, Deroee AF, Ebrahimkhani M, Kiani S, Dehpour A. Nitric oxide: a new concept in chronic sinusitis pathogenesis. *Am J Otolaryngol Head Neck Med Surg*. 2007; 28:334.
10. Ragab SM, Lund VJ, Saleh HA, Scadding G. Nasal nitric oxide in objective evaluation of chronic rhinosinusitis therapy. *Allergy*. 2006; 61:717. [PubMed: 16677241]
11. Benson, VM.; MA. National Center for Health Statistics. Current Estimates from the National Health Interview Survey 1995. 1998.
12. Adams, PF.; Hendershot, GE.; Marano, MA. National Center for Health Statistics. Current Estimates from the National Health Interview Survey 1996. 1999.
13. Thomas C, Darley-USmar V. Biological Chemistry and Clinical Potential of S-nitrosothiols. *Free Radical Biol Med*. 2000; 28:1478. [PubMed: 10927172]
14. Wang PG, Xian M, Tang X, Wu X, Wen Z, Cai T, Janczuk AJ. Nitric oxide donors: Chemical activities and biological applications. *Chem Rev*. 2002; 102:1091. [PubMed: 11942788]
15. Brisbois EJ, Handa H, Major TC, Bartlett RH, Meyerhoff ME. Long-term nitric oxide release and elevated temperature stability with S-nitroso-N-acetylpenicillamine (SNAP)-doped Elast-eon E2As polymer. *Biomaterials*. 2013; 34:6957. [PubMed: 23777908]
16. Fontijn A, Sabadell A, Ronco R. Homogeneous chemiluminescent measurement of nitric oxide with ozone. Implications for continuous selective monitoring of gaseous air pollutants. *Anal Chem*. 1970; 42:575.
17. Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J*. 1993; 6:1368. [PubMed: 7507065]
18. Jorissen M, Lefevre L, Willems T. Nasal nitric oxide. *Allergy*. 2001; 56:1026. [PubMed: 11703214]
19. Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations. *Eur Respir J*. 1997; 10:1683. [PubMed: 9230267]
20. GE Water & Process Technologies. Sievers Nitric Oxide Analyzer NOA 280i: Operation and Maintenance Manual. GE Analytical Instruments. 2010:11.
21. Shibuki K. An electrochemical microprobe for detecting nitric oxide release in brain tissue. *Neurosci Res*. 1990; 9:69. [PubMed: 2175870]
22. Shibuki K. Detection of Nitric Oxide by an Electrochemical Microprobe. *Neuroprotocols*. 1992; 1:151.
23. Shibuki K, Okada D. Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. *Nature*. 1991; 349:326. [PubMed: 1702879]
24. Jensen GC, Zheng Z, Meyerhoff ME. Amperometric nitric oxide sensors with enhanced selectivity over carbon monoxide via platinum oxide formation under alkaline conditions. *Anal Chem*. 2013; 85:10057. [PubMed: 24067100]

25. Malinski T, Taha Z. Nitric oxide release from a single cell measured in situ by a porphyrinic-based microsensor. *Nature*. 1992; 358
26. Malinski T, Taha Z, Grunfeld S, Burewicz A, Tomboulia P, Kiechle F. Measurements of nitric oxide in biological materials using a porphyrinic microsensor. *Anal Chim Acta*. 1993; 279:135.
27. Chen L, Tsang SC. Ag doped WO₃-based powder sensor for the detection of NO gas in air. *Sensors Actuators B: Chem*. 2003; 89:68.
28. Tomchenko AA, Khatko VV, Emelianov IL. WO₃ thick-film gas sensors. *Sensors Actuators B: Chem*. 1998; 46:8.
29. Wetchakun K, Samerjai T, Tamaekong N, Liewhiran C, Siri Wong C, Kruefu V, Wisitsoraat A, Tuantranont A, Phanichphant S. Semiconducting metal oxides as sensors for environmentally hazardous gases. *Sensors Actuators B: Chem*. 2011; 160:580.
30. Ho K, Hung W, Yang J. On the Electrooxidation and amperometric detection of NO gas at the Pt/Nafion electrode. *Sensors*. 2003:290.
31. Jacquinet P, Hodgson A, Hauser P. Amperometric detection of NO and NO₂ in the ppb range with solid-polymer electrolyte membrane supported noble metal electrodes. *Anal Chim Acta*. 2001; 443:53.
32. Knake R, Jacquinet P, Hodgson AWE, Hauser PC. Amperometric sensing in the gas-phase. *Anal Chim Acta*. 2005; 549:1.
33. Opekar F, Štulík K. Electrochemical sensors with solid polymer electrolytes. *Anal Chim Acta*. 1999; 385:151.
34. Hodgson AWE, Jacquinet P, Jordan LR, Hauser PC. Amperometric Gas Sensors of High Sensitivity. *Electroanalysis*. 1999; 11:782.
35. Jordan LR, Hauser PC, Dawson GA. Amperometric Sensor for Monitoring Ethylene. *Anal Chem*. 1997; 69:558.
36. Ho K, Liao J, Yang C. A kinetic study for electrooxidation of NO gas at a Pt/membrane electrode-application to amperometric NO sensor. *Sensors Actuators B: Chem*. 2005; 108:820.
37. Ren H, Wu J, Xi C, Lehnert N, Major T, Bartlett RH, Meyerhoff ME. Electrochemically modulated nitric oxide (NO) releasing biomedical devices via copper(II)-tri(2-pyridylmethyl)amine mediated reduction of nitrite. *ACS Appl Mater Interfaces*. 2014; 6:3779. [PubMed: 24611831]
38. Fedkiw PS. An Impregnation-Reduction Method to Prepare Electrodes on Nafion SPE. *J Electrochem Soc*. 1989:899.
39. Ho K, Hung W. An amperometric NO₂ gas sensor based on Pt/Nafion Electrode. *Sensors Actuators B: Chem*. 2001; 79:11.
40. The Neonatal Inhaled Nitric Oxide Group. Inhaled Nitric Oxide in Full-Term and Nearly Full-Term Infants with Hypoxic Respiratory Failure. *N Engl J Med*. 1997; 336:597. [PubMed: 9036320]
41. Rossaint R, Falke KJ, Lopez F, Slama K, Pison U, Zapol WM. Inhaled Nitric Oxide for the Adult Respiratory Distress Syndrome. *N Engl J Med*. 1993; 328:399. [PubMed: 8357359]
42. Doña Rodríguez JM, Herrera Melián JA, Pérez Peña J. Determination of the Real Surface Area of Pt Electrodes by Hydrogen Adsorption Using Cyclic Voltammetry. *J Chem Educ*. 2000; 77:1195.
43. Yamaya M, Sekizawa K. Increased carbon monoxide in exhaled air of subjects with upper respiratory tract infections. *Am J Respir Crit Care Med*. 1998:1.
44. McClure SM, Goodman DW. New insights into catalytic CO oxidation on Pt-group metals at elevated pressures. *Chem Phys Lett*. 2009; 469:1.
45. Sobkowski J, Czerwinski A. Voltammetric Study of CO and CO₂, Adsorption on Smooth and Platinized Platinum Electrodes. *J Phys Chem*. 1985; 89:365.
46. Dahlström PK, Harrington DA, Seland F. Kinetic study of CO oxidation on clean and oxidized Pt. *Electrochim Acta*. 2012; 82:550.

Highlights

A sensitive amperometric NO gas sensor based on Pt-Nafion electrodes is reported

Selectivity over CO is > 100-fold after conditioning the sensor in 10 ppm CO

The sensor can accurately measure NO released from NO-donor doped biomaterials

Good correlation is found via measurements using the sensor and chemiluminescence

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Figure 1. Schematic of amperometric SPE-based NO Sensor. WE = Pt-Nafion; CE = bare Pt; RE = single-junction Ag/AgCl in saturated KCl; internal electrolyte = 0.5 M H₂SO₄.

Figure 2. Sensing configuration employed for the determining the rates of NO release from SNAP/Carbosil2080A films by photolysis reaction at different intensities of light.

Figure 3. Sensing configuration employed for determining the rate of NO generated using electrochemical reduction of nitrite via use of CuTPMA as the electron transfer mediator.

Figure 4.

Representative amperometric response of Pt-Nafion sensor to NO standard gas (in N₂ background) in 200 ppb increments. A constant total flow rate of 50 mL min⁻¹ of the standard gas mix was used. Linear regression of calibrations with error (n = 5) is shown in the inset.

Figure 5. Selectivity of Pt-Nafion sensor vs. CO as a function of conditioning time in presence of 10 ppm CO at a flow rate of 50 mL min^{-1} ($n = 3$).

Figure 6.

(a) Amperometric Pt-Nafion sensor response to photolysis induced NO release from SNAP/Carbosil2080A films at varying light intensities. (b) Chemiluminescence response to NO released from SNAP/Carbosil2080A films at varying light intensities. The same light intensity settings were used for both techniques.

Figure 7.

(a) Amperometric Pt-Nafion sensor response to electrochemically generated NO formed via the CuTPMA-mediated reduction of nitrite ions in solution at various applied cathodic potentials in 4 mL of solution containing 1 M NaNO_2 and 0.3 M CuTPMA. The working electrode was a Teflon-coated Pt-Ir alloy wire with an exposed surface area of 0.08 cm^2 (b) Chemiluminescence response to NO released from the same solution described in (a). The same potentials were used for both gas phase measurement techniques.