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Amperometric Nitric Oxide Sensors with Enhanced Selectivity Over Carbon Monoxide via Platinum Oxide Formation Under Alkaline Conditions

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Abstract

An improved planar amperometric nitric oxide (NO) sensor with enhanced selectivity over carbon monoxide (CO), a volatile interfering species for NO sensors that has been largely overlooked until recently, is described. Formation of an oxide film on the inner platinum working electrode via anodic polarization using an inner alkaline electrolyte solution provides the basis for improved selectivity. Cyclic voltammetry reveals that formation of oxidized Pt film inhibits adsorption of CO to the electrode surface, which is a necessary initial step in the electrocatalytic oxidation of CO on Pt. Previous NO gas sensors that employ internal electrolyte solutions have been assembled using acidic internal solutions, that inhibit the formation of a dense platinum oxide film on the working electrode surface. It is demonstrated herein that increasing the internal electrolyte pH promotes oxidized platinum film formation, resulting in improved selectivity over CO. Selectivity coefficients ($\log K_{NO,i}$) for sensors assembled with internal solutions at various pH values range from -0.08 at pH 2.0 to -2.06 at pH 11.7 with average NO sensitivities of $1.24 \text{ nA}/\mu\text{M}$ and LOD of $<1 \text{ nM}$.

Keywords

amperometric gas sensor; nitric oxide; carbon monoxide; platinum oxide; adsorption

INTRODUCTION

The discovery of nitric oxide (NO) as the endothelial-derived relaxing factor in 1987^{1,2} has lead to a proliferation in research of its many physiological functions. In addition to its role as a smooth-muscle relaxer and vasodilator³, NO has been implicated in neurotransmission⁴, inhibition of platelet activation and adhesion⁵, and as a potent antimicrobial agent⁶. Nitric oxide is generated endogenously during the conversion of L-arginine to L-citrulline via heme-based enzymes known as NO synthases⁷. Gas-phase NO levels in exhaled oral and nasal breath may be diagnostically useful for detecting asthma⁸ and sinus infections⁹, respectively. In the latter case, it is now recognized that NO, given its high concentrations within the para-sinus cavities ($>100 \text{ ppb}$)⁹, serves as a potent antimicrobial and antifungal agent to decrease the occurrence of both sinus and lung infections.

SUPPORTING INFORMATION

Supporting Information Available: This material is available free of charge via the Internet at <http://pubs.acs.org>.

Due to the importance of physiological NO, there is demand for effective quantitative methods for NO detection. While a number of techniques have been developed to quantify NO levels, most rely on indirect routes such as the Griess assay¹⁰, which measures nitrite spectroscopically after NO oxidation in oxygenated solutions, or by measuring methemoglobin after NO reaction with oxyhemoglobin¹¹. The most direct methods for NO measurement are currently chemiluminescence^{12,13} and electrochemical techniques. While chemiluminescence offers very high sensitivity and selectivity¹⁴, it is expensive, has poor spatial resolution, and suffers from excessive foaming when used to analyze samples rich in protein. A review by Hetrick and Schoenfisch¹⁴ offers a detailed summary of currently available techniques for NO measurement.

Electrochemical measurement of NO via oxidation to nitrate offers a number of attractive advantages over other methods. Amperometric sensors have very low limits of detection (1–10 nM in many cases)^{14–18}, good selectivity over many potential interfering species, such as nitrite and ascorbate^{14–18}, and can be miniaturized, granting spatial resolution for detection of local concentrations of NO^{16,19,20} that enables NO imaging^{21,22}. A variety of amperometric NO sensors have been developed over the last twenty years, although most are based on either the Clark-type sensor configuration first suggested by Shibuki^{17,23} or the solid microsensor scheme originally described by Malinski^{16,20}.

Due to the high oxidation potentials required for NO oxidation (e.g., 0.7–0.9 V vs. Ag/AgCl)^{16, 23}, reducing electrochemical interference has become a recent focus in amperometric NO sensor design and development. Exclusion of ionic species that oxidize directly on the working electrode surface can be achieved through applying perm-selective coatings^{14,16,18,24} or gas-permeable membranes^{15,23} to the sensor assembly, however, volatile species, such as carbon monoxide (CO), remain problematic. Depending on the sample type, volatile interfering species can be a significant obstacle in the acquisition of an accurate analytical NO measurement.

Carbon monoxide is a common interfering species in a number of physiological applications of NO sensors, especially for the measurement of NO in exhaled oral²⁵ and nasal air^{25,26}, as well as various tissues that liberate NO. Recently, several studies have focused on overcoming this CO interference problem with amperometric NO sensors. For example, Shim *et al.* have developed a xerogel solid microsensor²² with CO sensitivity less than 5% of that for NO while Park *et al.* have developed a Shibuki style sensor by co-depositing Fe(III) nanoparticles and Pt black on the Pt working electrode to achieve enhanced selectivity for NO over CO²⁷. These efforts to discriminate CO in amperometric NO sensors are effective to varying degrees, however, fabrication of such sensors can be complex or lack structural stability.

We describe in this report a Shibuki-style amperometric NO sensor based on a Pt working electrode that exhibits improved selectivity over CO as a function of the internal electrolyte pH employed to fabricate the sensor and/or the solution used to initially polarize the Pt working electrode. Using a previously reported sensor design from our group^{15,19}, we examine the effect of internal electrolyte solution pH on the observed selectivity for NO over CO. Almost all Shibuki-type NO sensors reported to date, including the original version of our design, employ acidic internal electrolytes (pH 0–2) that form the layer between the working Pt electrode and the gas permeable membrane of the device. Herein, we examine the effect of increasing the pH to neutral and alkaline conditions on the performance and selectivity of this NO sensor configuration. It will be shown that use of a higher pH inner-filling solution increases the formation of a stable platinum oxide film on the working electrode surface, that serves to significantly decrease the oxidation of CO at the inner electrode surface without changing the efficiency of NO oxidation, thereby

substantially increasing selectivity of the sensor over CO. Further, this selectivity enhancement is preserved if the oxide layer is first formed under alkaline conditions prior to sensor assembly with a neutral pH internal solution.

EXPERIMENTAL SECTION

Chemicals and Reagents

Platinum wire (diameter 76 μm) and Ag wire (diameter 250 μm) were purchased from Sigma-Aldrich (St. Louis, MO). Borosilicate glass capillaries (outer diameter 2 mm, inner diameter 1.12 mm, length 100 mm) were obtained from World Precision Instruments Inc. (Sarasota, FL). Microporous poly(tetrafluoro-ethylene) (PTFE) membranes (Tetratex®, pore size 0.07 μm , thickness ~18 μm) were from Donaldson Company, Inc. (Minneapolis, MN). Teflon AF® (Grade 601-100-6) was obtained from DuPont Fluoroproducts (Wilmington, DE).

Nitric oxide and CO-saturated stock solutions were prepared by bubbling deionized water with pure NO and CO gases in an airtight vessel. Gas-phase NO was generated from acidification of NaNO_2 by H_2SO_4 and scrubbed of N_2O by sparging through 5% sodium hydroxide, all purchased from Sigma-Aldrich (St. Louis, MO). Carbon monoxide gas was obtained from Matheson Tri-Gas (Albuquerque, NM).a

Platinizing solution (3% chloroplatinic acid, <0.1% lead acetate, water balance) was purchased from LabChem Inc. (Pittsburgh, PA). Trizma base, H_2SO_4 , KCl, NaH_2PO_4 , and Na_2HPO_4 , $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, HCl, and NaOH were products of Sigma-Aldrich. All solutions were prepared with 18 M $\Omega\text{-cm}$ deionized water using reagent grade compounds without further purification.

Fabrication of Clark-type NO Sensors

The amperometric NO sensor was prepared as described previously¹⁵ and is depicted in Figure 1. Briefly, a 76 μm Pt wire was sealed in a glass capillary and filed on one end with 600 grit carbide to expose a planar Pt disk. The Pt disk was polished with 0.3 μm alumina and rinsed with deionized water. The Pt disk was then platinized by cycling from +0.6 V to -0.3 V at 20 mV/s for 2 cycles in platinizing solution. The disk electrode was rinsed thoroughly in deionized water following platinization. The Ag/AgCl reference electrode was prepared by taking a coiled 250 μm Ag wire and cycling from 0 to 0.5 V at 100 mV/s for 30 cycles in 0.1 M HCl.

The platinized Pt disk electrode and Ag/AgCl coil reference electrode were then inserted into the sensor housing (sealed with Teflon® AF treated PTFE prior to assembly for enhanced ammonia selectivity²⁸) along with the internal electrolyte (10 mM PBS, 140 mM KCl, titrated pH 7.4 and 11.7, 10 mM BBS titrated to pH 9, unbuffered 0.01 M HCl for pH 2, or unbuffered 0.5 M H_2SO_4 for pH 0). Sensors were then polarized in pH 7.4 PBS solution for at least 6 h at 0.7 V vs. Ag/AgCl prior to amperometric measurements. Measurements and calibrations were performed by injecting sample or standard into a pH 7.4 PBS buffer solution, with stirring, and allowing the current to reach a steady state.

Cyclic Voltammetry of Pt Electrode Surface

All CV experiments were performed in N_2 -purged pH 2.0 (10 mM HCl, 130 mM KCl) and pH 11.7 (10 mM PBS, 138 mM NaCl, 2 mM KCl) solutions using clean and oxidized Pt surfaces. The oxidized surface was prepared by taking a platinized Pt electrode and applying a constant potential of +0.7 V in the internal solution being investigated. Cyclic voltammetry measurements involved cycling from -0.6 to 0.8 V in pH 11.0 internal solution

and from -0.1 to 1.1 V in pH 2.0 internal solution at 10 mV/sec. For CO stripping experiments, CO was adsorbed onto the working electrode by immersing the Pt disk electrode for 10 min in a CO-saturated internal solution of the type under investigation.

RESULTS AND DISCUSSION

Development of a Reversible Amperometric NO Sensor Selective Against CO

Amperometric signals of NO sensors in response to standard additions of CO and NO are shown in Figure 2 for the Shibuki style sensors assembled using pH 2.0 and pH 11.7 internal solutions. There is little difference in NO sensitivity between sensors assembled using acidic vs. basic inner solutions; however, there is significantly lower response to CO from sensors assembled using a basic internal solution, suggesting improved selectivity over CO. Selectivity is defined by the following equation¹⁸:

$$\log K_{\text{NO},j} = \log \left(\frac{\Delta I_j / c_j}{\Delta I_{\text{NO}} / c_{\text{NO}}} \right)$$

here I_{NO} and I_j are the measured currents for NO and interfering species (in this case, $j = \text{CO}$) and c_{NO} and c_j are the concentrations of NO and interfering species, respectively.

Similar experiments were also conducted using a series of internal solutions (pH 0, 2.0, 7.4, 9.0, and 11.7). A summary of the selectivity coefficients determined as a function of internal solution pH is shown in Figure 3. Selectivity against CO is improved substantially as a function of increasing internal electrolyte solution pH. Based on the amperometric data, it is clear that enhanced selectivity for NO over CO can be achieved by increasing the alkalinity of the internal electrolyte solution. This sensor modification allows for the composition of the outer gas permeable membrane to remain unchanged, retaining the selectivity over other common interfering species in biological samples, such as nitrite, ascorbate and ammonia²⁸. Any differences in the response times of the sensors are primarily due to irreproducibility in the thickness of the porous Teflon membrane® or the thickness of the inner electrolyte layer between the platinum working electrode and this Teflon outer membrane.

A secondary goal for this work was to investigate how use of a high pH internal electrolyte solution improves selectivity for NO over CO. We hypothesize that an increase in the internal electrolyte pH may lead to greater oxidized Pt coverage on the inner working electrode surface when polarized at oxidizing potentials, passivating the surface towards CO oxidation. This behavior has been previously suggested by Tsceng and Yang²⁹ in their development of a CO sensor and later observed by Ho *et al.*³⁰ in the development of a solid polymer electrolyte based NO sensor for kinetic studies. This phenomenon was not previously investigated in detail and was offered only as speculation in this earlier work. In 1957, Anson *et al.* found that Pt electrodes, while generally considered “noble”, form oxide films³¹ under sufficiently oxidizing potentials, even under acidic conditions. A number of subsequent studies have since confirmed this finding, although the exact composition of the oxide film remains unclear³².

In a bimolecular surface reaction, such as the oxidation of CO to CO₂ on Pt, the two major possible reaction mechanisms are the Langmuir-Hinshelwood (LH) mechanism, where two reactants adsorb independently and subsequently react on the surface, and the Eley-Rideal (ER) mechanism, where one of the reactants adsorbs to the surface while the other reacts with the adsorbed species without itself adsorbing. In both the LH and ER mechanisms, CO must be adsorbed to the electrode surface for oxidation to proceed, and the dominant mechanism for CO oxidation on Pt is generally accepted to be an LH mechanism³³. In either

case, CO must be capable of adsorbing to the electrode surface in order for oxidation to occur. There is a lack of previous work relating to CO adsorption and subsequent oxidation on electrochemically oxidized polycrystalline Pt. Investigations of CO adsorption and oxidation in the literature have been primarily performed using single-crystal Pt³⁴.

We hypothesize that the increased pH of the internal solution results in formation of a more extensive film of oxidized platinum during polarization of the sensor when a constant anodic potential of +0.7 V vs. Ag/AgCl is applied for long periods of time. Pourbaix diagrams for polycrystalline Pt also suggest that pH has a profound effect on the formation and composition of oxidized platinum films, with increasing pH favoring oxide formation³⁵. It is conceivable that greater coverage of oxidized Pt on the electrode surface may prevent adsorption of CO, hence passivating the surface towards CO oxidation. Thus, clean and oxidized Pt electrode surfaces were placed in bulk “internal” solutions of pH 11.7 and pH 2.0 and oxidatively stripped of previously adsorbed CO in order to probe the behavior of the electrode surface in acidic and basic environments. Corresponding CV's for NO oxidation at clean and oxidized Pt surfaces may also be useful, although it has been shown that NO does not adsorb to a great extent on polycrystalline platinum^{33,36}. Instead, NO oxidation is suggested to occur primarily in bulk solution and not as an adsorbate, making adsorption an unnecessary step in the overall mechanism³⁶. Furthermore, the oxidation of NO appears to be largely independent of the electrode material, suggesting weak interactions with the electrode surface.

In order to elucidate the mechanism of inhibition of electrochemical CO oxidation, cyclic voltammetry was employed to probe the behavior of CO at platinum electrode surfaces. Background CV's are shown in Figure 4 with oxide reduction from -0.2 to -0.4 V in pH 11.7 solution and oxide reduction from +0.3 to +0.5 V in pH 2.0 solution. Note that no oxide reduction occurs on the initial negative sweep, confirming that the reduction peak on the second cycle is likely the oxide formed during the anodic sweep. The oxide formed also appears more extensive when the Pt electrode is oxidized under basic conditions, as hypothesized, with an 11-fold increase in total charge passed during reduction (molar quantity cannot be determined due to ambiguity of the exact oxide layer composition).

Carbon monoxide oxidation occurs over a potential range of +0.45 to +0.5 V in pH 2.0 solution and -0.2 to -0.3 V in pH 11.7 solution, as shown in Figure 5. Under acidic conditions, there is little difference in the CO adsorption and oxidation behavior between a clean Pt surface and one that had been pre-oxidized. Under basic conditions, however, there is no CO stripping current during the initial positive sweep for the oxidized Pt, suggesting that CO does not adsorb to the oxidized electrode surface, while a very clear feature is seen for the clean Pt surface. Without adsorption to the surface, it is unlikely that CO would be oxidized to a great extent via a bimolecular surface reaction.

Nitric oxide adsorption on polycrystalline Pt has been attempted by previous investigators without consistent observation of an adsorbed species³⁶. It has been suggested that NO adsorption is not critical to NO oxidation in bulk aqueous solutions³⁶.

If selectivity is pH-dependent and the improved NO sensors are to be used in biomedical applications, such as detection of NO in exhaled nasal breath, exposure to high levels of CO₂ may titrate the internal solution between the working electrode and the gas permeable membrane. Due to the small volume of this layer and the elevated quantities of CO₂ present, the pH of this alkaline layer is titrated to pH 6.4 within 25 min (see Figure S-1) in a sample solution when bubbling 10% CO₂. However, the formation of the oxide layer, responsible for the selectivity of the sensor over CO, occurs rapidly before the drop in pH occurs and appears to be stable at pH 6.4. Thus, the response to NO is not compromised and the

selectivity of the sensor remains intact even after exposure to high CO₂ levels (see Figure S-3).

CONCLUSIONS

An amperometric, planar NO sensor with high sensitivity for NO and high selectivity over CO has been developed and characterized. The sensor is a Shibuki-type device with internal filling solution and internal Ag/AgCl reference electrode housed with a platinum working electrode behind a gas permeable membrane. We conclude that selectivity over CO in such a sensor configuration can be achieved, without compromising sensitivity towards NO, by elevating the internal solution pH. A basic internal solution lowers the potential for forming an oxidized Pt layer on the Pt working surface, that inhibits CO adsorption as shown via cyclic voltammetry. Carbon monoxide adsorption is observed on clean Pt surfaces as well as surfaces oxidized in the traditional acidic internal solution, while such adsorption is inhibited on a Pt surface pre-oxidized in a basic internal solution. Carbon monoxide oxidation on a Pt surface requires CO adsorption to the electrode surface; the oxidized Pt layer is responsible for greatly reducing CO interference in the proposed sensor configuration. By using a basic internal solution, a more extensive oxidized Pt layer is formed on the working electrode surface, which precludes CO adsorption to a significant extent. It is likely that this new improved-selectivity NO sensor can be readily applied for biomedical measurements, such as measurement of NO released from explanted tissues and exhaled nasal breath, where CO interference was previously an obstacle.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Schematic of the planar, amperometric nitric oxide sensor used in this study.

Figure 2. Amperometric response to standard additions of (A) NO and (B) CO of sensors assembled with pH 11.7 (red) and pH 2.0 (black) internal filling solution (10 mM PBS, 138 mM NaCl, 2 mM KCl)

Figure 3.
 $\log K_{\text{NO}_j}$ of sensors assembled using internal solutions of varying pH.

Figure 4. Background cyclic voltammograms of clean (red) and oxidized (black) Pt disk electrodes in (A) pH 2.0 solution and (B) pH 11.7 solution.

Figure 5. CO stripping cyclic voltammograms of clean (red) and oxidized (black) Pt disk electrodes in (A) pH 2.0 solution and (B) pH 11.7 solution.