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Nitric oxide permselectivity in electropolymerized films for sensing applications

Micah D. Brown, Mark H. Schoenfisch*

Department of Chemistry, University of North Carolina at Chapel Hill, CB 3290, Chapel Hill, NC 27599

Abstract

The presence of biological interferents in physiological media necessitates chemical modification of the working electrode to facilitate accurate electrochemical measurement of nitric oxide (NO). In this study, we evaluated a series of self-terminating electropolymerized films prepared from one of three isomers of phenylenediamine (PD), phenol, eugenol, or 5-amino-1-naphthol (5A1N) to improve the NO selectivity of a platinum working electrode. The electrodeposition procedure for each monomer was individually optimized using cyclic voltammetry (CV) or constant potential amperometry (CPA). Cyclic voltammetry deposition parameters favoring slower film formation generally yielded films with improved selectivity for NO over nitrite and L-ascorbate. Nitric oxide sensors were fabricated and compared using the optimized deposition procedure for each monomer. Sensors prepared using poly-phenol and poly-5A1N film-modified platinum working electrodes demonstrated the most ideal analytical performance, with the former demonstrating the best selectivity. In simulated wound fluid, platinum electrodes modified with poly-5A1N films proved superior with respect to the NO sensitivity and detection limit.

Graphical Abstract

*Corresponding Author schoenfisch@unc.edu.

The corresponding author declares the following competing financial interest: Mark Schoenfisch is a founder of and maintains a financial interest in Clinical Sensors, Inc. Clinical Sensors is an early-stage medical device company developing sensors for use in hospital settings.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. Selectivity coefficients for NO under different electrodepositional techniques/parameters and differential pulse voltammograms of NO on bare Pt, poly-phenol-, and poly-5A1N-modified electrodes (PDF).

Keywords

nitric oxide; permselectivity; phenylenediamine; phenol; eugenol; 5-amino-1-naphthol; electropolymerization; polymer-modified electrode

Nitric oxide (NO) is a gaseous free radical endogenously produced from L-arginine by a group of isoenzymes called nitric oxide synthase (NOS).^{1,2} Much prior work has revealed the importance of NO for essential physiological processes including wound healing,³ angiogenesis,^{4,5} platelet aggregation,⁶ vasodilation, inflammation, and the immune response.⁷ As the significance of this small molecule continues to be understood, the demand for accurate detection in biological systems grows as well. Due to its short half-life in the presence of various scavengers (oxygen, peroxides, free metals, and hemoglobin^{8,9}), real-time measurements of NO in biological media are analytically challenging.¹⁰ Although direct and sensitive detection can be achieved utilizing chemiluminescence or electron paramagnetic resonance (EPR), these techniques require complex, expensive instrumentation. Furthermore, these methods do not facilitate measurement in complex milieu such as blood or wound fluid.^{11,12} In contrast, electrochemical strategies allow for real-time monitoring of NO in a simple, fast, and low-cost manner with superior spatial and temporal resolution.¹³⁻¹⁵

The sensitivity, dynamic range, and limit of detection (LOD) of an electrochemical sensor are important properties given the varied, but generally small concentrations of NO that exist physiologically. An equally important consideration is selectivity, particularly for amperometric measurements. Interfering species such as L-ascorbate, nitrite, acetaminophen, and dopamine are common in biological milieu and pose a significant obstacle to accurate NO measurement.^{16,17} These interferents are oxidized at working potentials similar to or below those required to detect NO, and therefore contribute erroneously to the current response. In order to preserve sensor accuracy, the predominant strategy is to employ one or more membranes over the working electrode that selectively permit permeation of NO while reducing or eliminating the diffusion of interferents. Sensors that reduce interferent passage relative to that of the target analyte are said to have high permselectivity. Membranes used to fabricate NO sensors in this manner include Nafion,^{18,19} Teflon,^{20,21} nitrocellulose,²² chloroprene,²³ and xerogel monoliths.²⁴ Greater hydrophobicity, size-exclusion, and/or charge-repulsion are often cited as the primary interferent exclusion mechanisms for these

membranes. While enhanced NO selectivity is often observed, the depositions of these polymers necessitates solvent casting, resulting in undesirable variability in film thickness and, consequently, poor control over both NO sensitivity and selectivity.²⁵

Self-terminating electropolymerized films represent a class of sensor membranes characterized by reproducible and straightforward depositions. Through electrooxidation-initiated radical coupling reactions, monomers polymerize and precipitate onto the working electrode surface. Growth of the film eventually creates an insulating layer that prohibits further monomer access to the electrode, resulting in depositions that are self-assembled and finite. The thickness of self-terminating films formed by electropolymerization spans 5–35 nm.^{26–29} The films are deposited onto the electroactive surface with high specificity (i.e., without the need for masking) and uniformity regardless of electrode size or geometry,³⁰ features that make electropolymerized films a compelling choice in fabricating sensors with consistent performance. A number of aromatic monomers have been used to create electropolymerized films for selective NO detection, often in conjunction with a secondary or tertiary membrane (e.g., Nafion).^{31,32} Among the most frequently employed polymer films are those prepared from the three isomers of phenylenediamine (*o*-, *m*-, and *p*-PD), phenol, eugenol, and 5-amino-1-naphthol (5A1N).^{30,33} While prior work has largely investigated the potential of these films for hydrogen peroxide (H₂O₂) permselectivity,^{25,27,34,35} studies with NO as the focus analyte have yet to be carried out.

Secondary to monomer selection, the electrochemical deposition technique may influence oligomer structure, and consequently the film morphology and permeability.^{36,37} Indeed, the effects of monomer deposition conditions are often neglected with respect to permselectivity. Herein, we set out to evaluate the NO selectivity and analytical performance characteristics of sensors prepared using self-terminating electropolymerized films as a function of both monomer selection and deposition procedure. The objective of this work was to ascertain optimal electropolymerization methods for each monomer individually and then compare ensuing sensor performance as a function of monomer identity.

Experimental Section

Materials and reagents

Ortho-phenylenediamine (*o*-PD), *meta*-phenylenediamine (*m*-PD), *para*-phenylenediamine (*p*-PD), phenol, eugenol, 5-amino-1-naphthol (5A1N), hydrochloric acid (HCl), sodium nitrite, L-ascorbic acid, acetaminophen, dopamine hydrochloride, and fetal bovine serum (FBS) were purchased from Sigma (St. Louis, MO). Ammonium hydroxide, sodium hydroxide (NaOH), sulfuric acid, and hydrogen peroxide (30 wt%) were obtained from Fisher Scientific (Hampton, NH). Nitric oxide gas (99.5%) was purchased from Praxair (Danbury, CT). Nitrogen (N₂) and argon (Ar) gases were purchased from National Welders Supply (Raleigh, NC). Other solvents and chemicals were analytical-reagent grade and used as received without further purification.

A Millipore Milli-Q UV Gradient A10 Water Purification System (Bedford, MA) was used to purify distilled water to a final resistivity of 18.2 MΩ-cm and a total organic content of 6 ppb. Saturated NO solutions (1.9 mM) were prepared by purging ~20 mL of phosphate

buffered saline (PBS; 10 mM, pH 7.4) with Ar for 30 min, followed by NO gas for 20 min over ice. Solutions were used the day of preparation and stored at 4 °C. Electrochemical experiments were carried out using a CH Instruments 920D Scanning Electrochemical Microscope (Austin, TX). The four-electrode arrangement employed two inlaid 2 mm diameter polycrystalline platinum (Pt) disc working electrodes sealed in Kel-F (6 mm total diameter; CH Instruments), a silver-silver chloride (Ag|AgCl) reference electrode (3.0 M KCl; CH Instruments), and a coiled Pt wire counter electrode. All potentials herein are reported versus the Ag|AgCl reference electrode.

Preparation of the working electrode

Platinum disc working electrodes were mechanically polished on a microcloth with successively finer grades of deagglomerated alumina slurries down to 0.05 μm in particle size (Buehler; Lake Bluff, IL). Electrodes were rinsed with distilled water and ultrasonicated in ethanol to remove residual alumina loosely bound to the surface. The electrodes were then dried under a stream of N_2 and electrochemically polished in 1 N hydrosulfuric acid by cycling between -0.4 and $+1.8$ V for 20 cycles (500 mV s^{-1}). Additional cycling in PBS between 0 and $+1.0$ V (100 mV s^{-1}) was applied until steady state cyclic voltammograms resulted. Prior to deposition, Pt electrodes were calibrated using amperometry ($+0.8$ V) and injections of $1.9 \mu\text{M}$ saturated NO solution in deoxygenated PBS.

Electrodeposition of polymer films

The *o*-PD, *m*-PD, *p*-PD, and phenol monomers were dissolved in PBS at pH 7.4. For reasons of solubility, eugenol was dissolved in PBS titrated to pH 13 with NaOH. Likewise, 5A1N was dissolved in PBS titrated to pH 1 with HCl. The electropolymerization process was carried out through either cyclic voltammetry (CV) or constant potential amperometry (CPA). Cyclic voltammetric depositions were performed in either 10 or 100 mM solutions of monomer by sweeping the potential from 0 to $+1.0$ V (20 cycles; positive direction initial sweep) at a scan rate of either 10 or 100 mV s^{-1} . Electropolymerizations via constant potential amperometry were carried out for 15 min in either 10 or 100 mM solutions of monomer and an applied potential of $+0.6$, $+0.75$, or $+0.9$ V. In total, ten distinct electrodeposition procedures were evaluated for each monomer (with the exception of 5A1N, which was only soluble up to 10 mM). The resulting film-modified electrodes were then rinsed with distilled water to remove unbound oligomers prior to electrode testing.

Steady-state amperometric measurements

Film-modified Pt electrodes were polarized at $+0.8$ V in deoxygenated PBS for 10 min to achieve a constant background current prior to measurements. The change in electrooxidative current was then measured after injecting saturated NO solution aliquots ($1.9 \mu\text{M}$) to determine the change in sensitivity relative to bare Pt. Stock solutions of various interferents were initially prepared in PBS to test for selectivity to NO. The current responses to injections of sodium nitrite (1 mM), L-ascorbate (1 mM), acetaminophen (250 μM), dopamine hydrochloride (100 μM), ammonium hydroxide (1 mM), and hydrogen peroxide (1 mM) were measured and compared to the NO sensitivity. Selectivity coefficients were calculated using Eq. 1, where $\log k_{\text{NO},j}$ is the selectivity coefficient for NO over

interferent j , i_j is the current response to interferent j , C_j is the concentration of interferent j , and S_{NO} is the NO sensitivity of the film-modified electrode.

$$\log k_{NO,j} = \log \left(\frac{\Delta i_j / C_j}{S_{NO}} \right) \quad (\text{Eq. 1})$$

Sensor performance in simulated wound fluid

Upon determining the optimal electrodeposition parameters for each monomer, the sensitivity of the film-modified electrodes was tested in simulated wound fluid (SWF), a 10-fold dilution of FBS in deoxygenated PBS. After polarizing the electrode at +0.8 V for 10 min in SWF, the background current and responses to injections of saturated NO (1.9 μM) were measured and compared to trials in PBS. These measurements were repeated after 1 h immersion in the SWF without agitation.

Contact angle measurements

The 2 mm diameter Pt disc electrodes were too small for goniometric measurements, necessitating the fabrication of larger, planar electrodes. Briefly, silica microscope slides were masked with Kapton® polyimide tape to delineate an electrode geometry before oxygen plasma cleaning (100 W, 30 s). A 100 nm Pt layer with a 10 nm titanium (Ti) adhesion layer was deposited onto the silica substrate using a Kurt J. Lesker PVD 75 magnetron sputtering system (Clairton, PA). After removal of the mask, a band of epoxy-adhered silica was used to separate the working electrode area ($\sim 1 \text{ cm}^2$) from the potentiostat lead. Following electropolymerization, static contact angles were measured using a CAM 200 Optical Contact Angle Meter (KSV Instruments; Bridgeport, CT). Water droplets ($\sim 8 \mu\text{L}$) were placed in the center of the electrode plane, and measurements were averaged across four depositions of the same monomer to assess the hydrophobicity of the electropolymerized films.

Statistical analysis

The selectivity, NO permeability, limit of detection, and contact angle of the film-modified electrodes are presented as the mean $\pm$ standard error of the mean. Comparisons between groups of electrochemical deposition parameters were performed using a two-tailed t -test with $p < 0.05$ considered to be statistically significant.

Results and Discussion

The six monomers in this study undergo irreversible electrooxidation in aqueous media upon the application of a sufficiently positive potential. The phenylene diamine series is oxidized through one or both amine groups,³⁸ whereas phenol and eugenol are oxidized through the hydroxyl group. While 5A1N contains both amine and hydroxyl groups, previous work has indicated that electropolymerization occurs through the amine.³⁹ Both the electrolyte identity and concentration in the monomer solution have been shown to affect film permeability in electropolymerized films, presumably via ion entrapment during electropolymerization.⁴⁰ All monomers were therefore dissolved in 0.01 M phosphate

buffered saline (PBS) solutions to maintain a constant electrolyte concentration and facilitate steady-state measurements. Platinum was chosen as the working electrode due to the ubiquity of its use and known sensitivity towards NO.^{17,41}

Cyclic voltammetry of monomers

The shapes of the CV traces collected using a platinum working electrode in solutions of each monomer result from both the monomer redox activity and the insulating process that occurs during the electrodeposition of a film (Figure 1). Most film deposition occurs during the initial sweep, blocking further monomer access and causing the electrooxidative current to diminish rapidly. The CV traces become distorted as a result, obscuring higher potential wave features. This phenomenon was particularly evident in the traces of *o*-PD and eugenol (Figure 1A and 1E) with abrupt shoulders at +0.65 and +0.75 V, respectively. After the switching potential (+1.0 V), only minor reduction waves were observed in the return traces, indicative of irreversible oxidation of parent monomers. The cathodic feature in the return trace of poly-5A1N (Figure 1F) is attributed to the film's conductive properties. However, the polymerization mechanism of poly-5A1N is not autocatalytic and still proceeds to self-terminate, unlike other conducting films (e.g., poly-aniline).⁴² Monomer oxidation potentials were measured at the first peak present in the initial anodic sweep. The presence of multiple peaks in a given wave is attributed to secondary oxidations of radical cations and/or over-oxidation of the deposited film.^{27,43} As evident in Figure 1C, *p*-PD had the lowest oxidation potential (+0.20 V), indicating that it is the most easily oxidized monomer and presumably the fastest to polymerize. The oxidation potential for phenol appeared at +0.62 V (Figure 1D), making this monomer the most difficult to oxidize. The oxidation potentials of other monomers fell in between *p*-PD and phenol in the following order: *p*-PD < eugenol < *o*-PD < *m*-PD < 5A1N < phenol. Increasing the monomer concentration to 100 mM and/or scan rate to 100 mV s⁻¹ did not influence this order.

Electropolymerization of monomers via cyclic voltammetry

Twenty cycles of the same potential window (0 to +1.0 V) were applied to deposit a complete, self-terminating film on the surface of Pt disc electrodes. The switching potential at +1.0 V represented a significant overpotential to all monomers, allowing for cyclical generations of radical cations. While others have suggested that the switching potential can influence both the degree of oligomer conjugation and charge-state of the film,^{34,43} we did not seek to study this parameters. Rather, we assessed the influence of scan rate (10 and 100 mV s⁻¹) and monomer concentration (10 and 100 mM) on the NO permselectivity, capturing the range of deposition variables reported previously.^{27,30,44-47} The self-limiting behavior of these film depositions via CV is well understood. Briefly, growth of the insulating film effectively blocks monomer transport to the electrode surface, capping radical cation generation and the polymerization process. As a result, the anodic current decreases precipitously with successive cycles. For example, the oxidation peak for phenol at +0.62 V is reduced upon poly-phenol film formation after the first cycle (Figure 2). The absence of anodic wave features in subsequent cycles indicates that the majority of the deposition/insulation process has occurred by the second cycle. Likewise, the switching potential current decreased rapidly during the first 10 cycles, converging to approximately -0.75 μ A to mark film completion.

The above traits, characteristic of electrodeposition/insulation, were observed for each monomer studied. The rate at which the current diminished with each cycle, however, was distinct with respect to both the deposition procedure and monomer identity, signifying different rates of film formation. Based on the decrease in peak oxidation current between the first and second cycles, the rate of film formation trended as follows: eugenol > *o*-PD > phenol > *m*-PD > 5A1N > *p*-PD. With the exception of phenol and *p*-PD, this order mirrored that of the oxidation peaks, presumably because lower oxidation potentials allow monomers to actively polymerize during a greater portion of each potential sweep. The fact that *p*-PD films were the slowest to form is likely the result of poor film formation, as CV tends to yield only low molecular weight oligomers of *p*-PD.⁴⁸ The loss of these soluble oligomers from the electrode surface also accounts for the deepening reddish-brown hue of the monomer solution as the deposition proceeded.²⁹

Four different electropolymerization procedures were carried out for each monomer via CV by increasing the scan rate and/or monomer concentration by an order of magnitude. In theory, such changes should increase the rate of radical cation generation, affecting the organization and density of precipitated oligomers on the electrode surface. The initial cycle and resultant oxidation peak shifts for each of the four different deposition conditions carried out in phenol are provided in Figure 3. As expected for irreversible redox reactions, increasing the scan rate from 10 to 100 mV s⁻¹ led to increased peak current and shifted the primary oxidation peak to more positive potentials. Similar results were observed for each of the other monomers upon increasing the scan rate. Increasing the phenol concentration from 10 to 100 mM shifted the peak potential negatively regardless of scan rate (Figure 3), demonstrating faster film formation and accompanying electronic insulation. While a positive correlation between peak height and redox agent concentration was anticipated, no such dependence was observed here. Convolution of the electronic properties of the monomer and the self-limiting nature of these depositions represent a likely explanation for these results (as previously noted in Figure 1). Solutions of *m*-PD demonstrated similar behavior to phenol. Depositions of 10 mM *o*-PD, *p*-PD, and eugenol resulted in peak potentials and currents close or identical to those in 100 mM monomer solutions, indicating a negligible convolution effect. In this respect, the monomer concentration does not have an appreciable effect on the CV deposition rate for these monomers. Of note, monomer concentration was not studied for 5A1N due to limited solubility in the aqueous media.

Electropolymerization of monomers via CPA

Monomers were electropolymerized by CPA onto the surface of Pt electrodes as a function of monomer concentration (10 and 100 mM) and applied potential (+0.6, +0.75, and +0.9 V). Prior work suggests that a significant overpotential is required to yield optimally permselective films (with respect to H₂O₂).^{34,36} Therefore, each of the applied potentials were chosen to exceed the first oxidation potential of each monomer, with the exception of phenol's oxidation peak at +0.62 V. The potential was applied to the working electrode for 15 min, a period that is typical period for CPA depositions of electropolymerized films.^{25,27,49} Due to the self-sealing nature of this process, longer amperometric polymerization times are generally not useful for further improving permselectivity.⁵⁰ A greater overpotential resulted in more easily oxidized phenol monomers at the electrode surface,

with a concomitant increase in the initial current (Figure 4). This effect was most pronounced between +0.6 and +0.75 V, the former just below phenol's oxidation potential. Likewise, the initial current was elevated upon increasing the concentration from 10 to 100 mM phenol due to the greater flux of monomers to the electrode surface. This increase in initial current (with greater overpotentials and/or monomer concentration) was noted for each of the monomers investigated in our study.

Another feature of the amperograms was the rate of convergence to a steady current, marking when film-formation was nearly complete. In phenol, the rate of convergence increased with the applied potential, signifying faster film-formation at large overpotentials (i.e., +0.75 and +0.9 V; Figure 4). The same dependence was observed for *o*-PD, *p*-PD, and eugenol. However, this behavior was not observed with *m*-PD and 5A1N, in which the CPA depositions at +0.6 V converged most rapidly, followed by +0.9 and +0.75 V. In addition to facilitating monomer oxidation, the magnitude of the overpotential also impacts the size, charge-state, and solubility of resulting oligomers—factors that may, if not optimized appropriately, reduce packing efficiency and lower the rate of film formation, as may have been the case with *m*-PD and 5A1N. Constant potential amperometry depositions in 100 mM solutions of phenol were complete prior to those using 10 mM solutions (Figure 4). The greater flux of monomers to the electrode surface clearly impacted the rate of oligomer generation. This trend was also observed for *o*-PD and eugenol, but not *m*-PD and *p*-PD, which were characterized as having slower deposition rates at higher monomer concentration. Of note, the limited solubility of 5A1N precluded the study of its concentration on film-formation kinetics.

Analytical performance of film-modified electrodes

The electropolymerized film-modified electrodes were characterized with respect to NO selectivity over nitrite (NO_2^-) and L-ascorbate (AA), two biologically relevant interferents. At physiological pH, both molecules are negatively charged and coulombically drawn into positively charged films, such as those formed by oxidative electropolymerization.²⁷ Nitrite is a particularly challenging interferent because it is the primary oxidation product of NO and of similar size. In contrast, AA is larger but has an oxidation potential that is lower than NO. In serum, AA is present at larger concentrations (34–144 μM) than nitrite (< 20 μM).^{16,51} The amperometric response from film-modified electrodes was measured at +0.8 V following the addition of interferent and NO solutions into deoxygenated PBS. Selectivity coefficients were then calculated using this data (Figure S1). The optimal deposition procedure in each monomer was determined by the sum of anionic selectivity coefficients against nitrite and AA.

The best deposition procedures for each monomer and relevant analytical merits are provided in Table 1. For each monomer except phenol, the optimal CV deposition procedure yielded more selective films to NO than all CPA depositions tested. Of these monomers, all except *m*-PD formed more NO-selective films at the slower scan rate (10 mV s^{-1}), suggesting that slow, oscillatory generations and precipitations of oligomer units yield the most densely packed membranes. Of note, the cumulative anionic selectivity for NO under the optimal CPA deposition of phenol (100 mM, +0.9 V) was not different than the optimal

deposition by CV (10 mM, 100 mV s⁻¹). The fact that CV depositions of the phenylenediamine (PD) series yielded more selective films than via CPA depositions (Figure 5) stands in contrast to prior reports of PD film permselectivity.^{27,29} Of note, the target analyte in these former studies was hydrogen peroxide and the comparison between CPA and PD was made without optimization of the CV deposition parameters. Potentiodynamic electro-polymerization techniques (e.g., linear sweep, squarewave, and cyclic voltammetry) are thought to generate a greater density of nucleation sites on the electrode surface, improving both compactness and film adherence.^{52,53}

Additional analytical performance metrics were measured for optimal film-modified electrodes, allowing for comparison on the basis of monomer identity. Many diverse physiological species are electroactive at the potentials required for NO oxidation, thus motivating testing selectivity against a range of interferents. As analogs to the AA/nitrite anionic pair, dopamine (DA) and ammonium (NH₄⁺) represent large and small cationic interferents, respectively. Likewise, acetaminophen (AP) and hydrogen peroxide (H₂O₂) are large and small neutral interferents, respectively. Poly-*o*-PD and poly-*m*-PD modifications led to the greatest selectivity coefficients against AA (selectivity coefficients -3.5 ; Table 1). Poly-*m*-PD also provided the best selectivity against the large, hydrophobic molecule AP (-3.7 ± 0.1), indicative of superior interferent rejection on the basis of size-exclusion. Relative to the other monomers, however, the greater selectivity of *m*-PD films came at the expense of LOD (90 ± 40 nM) and NO permeability (0.13 ± 0.04). Poly-*p*-PD was the best film for restricting DA (-2.6 ± 0.2), which suggests that it is more positively charged and capable of cationic repulsion. Within the PD isomer series as a whole, better selectivity coefficients against AA and AP versus the smaller interferents (nitrite and H₂O₂) verifies that size-exclusion plays a large role in restricting interferent diffusion to the electrode. While poly-eugenol-modified electrodes had favorable selectivity regardless of the interferent identity, the accompanying reduced permeability to NO (0.01 ± 0.00) and concomitant increase in LOD (130 ± 70 nM) preclude use for sensor applications.

Poly-phenol- and poly-5A1N-modified sensors were the most sensitive to NO, yielding the lowest NO detection limits (6 ± 5 and 4 ± 3 nM, respectively). Of note, this response to NO exceeded that observed for bare Pt. Electropolymerized films have been shown previously to aid in electron transport and enhance hydrogen peroxide detection without an extraneous catalyst or mediator.⁵⁴ The oxidation potentials of NO measured via differential pulse voltammetry on bare Pt, poly-phenol- and poly-5A1N-modified working electrodes were found to be 728 ± 2 , 713 ± 2 , and 649 ± 4 mV, respectively (Figure S2). The reduced oxidation potential observed for poly-5A1N, in particular, strongly suggests an electrocatalytic mechanism facilitating electron transfer to the working electrode. The NO response amplification observed for poly-phenol and poly-5A1N modified electrodes represents a significant advantage over the other monomers, particularly when improvements in permselectivity so often come at the expense of sensitivity. Poly-phenol films performed best against nitrite with a selectivity coefficient of -4.0 ± 0.1 , representing a 10,000-fold greater current response to NO. To our knowledge, this selectivity against nitrite is unprecedented in the literature with respect to electrochemical NO-sensing electrodes modified solely by an electropolymerized film. Poly-phenol films also exhibit unprecedented selectivity against hydrogen peroxide (-2.2 ± 0.1), a surprise given their extensive use in

glucose biosensors. While the oxidation potential employed for glucose biosensors is often lower than for NO (+0.6 vs. +0.8 V), our findings indicate that poly-phenol films are better suited for electrochemical NO detection. Poly-5A1N modified sensors also demonstrated notable nitrite blocking with a selectivity coefficient of -3.5 ± 0.1 . In terms of overall analytical performance, the poly-phenol and poly-5A1N modifications proved superior over the other monomers.

Performance in simulated wound fluid

Electropolymerized film-modified electrodes are favorable as NO sensors given their aforementioned reproducibility, high selectivity, and ease of fabrication. However, a key obstacle to in situ, biological NO measurements is biofouling, specifically the accumulation of proteins on the transducer surface and concomitant degradation in analytical performance. While the anti-fouling properties of a sensor depend largely on the surface chemistry of the outermost coating, electropolymerized films (the PD isomers in particular) are most commonly utilized as inner coatings and have not been compared systematically for their biofouling resistance.⁵⁵ The analytical performance (i.e., LOD and sensitivity retention) of polymer-modified electrodes was therefore characterized in simulated wound fluid (SWF) in order to compare anti-fouling characteristics and assess potential application of electrodes modified by a single electropolymerized film to measurements in complex media.

The NO sensitivity of bare and film-modified Pt electrodes decreased upon exposure to SWF relative to measurements in PBS (Figure 5A). The reduction in sensitivity was attributed to both protein surface fouling (obstructing NO permeation) and anticipated scavenging of NO in proteinaceous media. It is worth noting that the NO sensitivity remained constant even after 1 h immersion in SWF (no stirring). Biofouling at the polymer film interface likely occurs quickly, allowing for short preconditioning times. *Ortho*- and *m*-PD retained sensitivity better than bare Pt with the lowest degree of uncertainty ($p < 0.01$). *Meta*-PD-modified electrodes, in particular, displayed the greatest anti-fouling character, retaining 84% of the NO response (sensitivity) after 1 h SWF exposure relative to bare Pt (only 12%). Reynolds et al. reported similar sensitivity retention for *m*-PD surface-modified glucose biosensors (H_2O_2) upon SWF immersion (3% w/v FBS in pH 7.4 PBS).³⁵ Diminished NO sensitivity in SWF was often accompanied by an increase in background noise, resulting in elevated LODs (Figure 5B). Although changes in LOD from PBS to SWF trials were not statistically significant for the film-modified electrodes, a general increase in both LOD value and variability was observed (with the exception of eugenol, for which the LOD was already elevated in PBS). *Ortho*-PD and 5A1N modified electrodes were characterized as having the lowest LODs and changes in LOD in SWF relative to PBS, suggesting that these films would have the greatest utility in detecting NO in wound fluid.

The primary thermodynamic force driving protein and surface interaction is hydrophobicity. Due to the large release in entropic energy when an ordered water layer is disrupted by protein adsorption, hydrophilic surfaces are thought to have greater anti-fouling character than their hydrophobic counterparts.⁵⁶ The hydrophobicity of the electropolymerized films was assessed using static contact angle measurements. As shown in Figure 5B and C, *o*-PD, eugenol, and 5A1N films proved to be less hydrophilic than the other films, but with LODs

least impacted by biofouling. Furthermore, *p*-PD and phenol experienced large reductions in NO sensitivity and increases in LOD (Figures 5A and 5B, respectively) despite being the most hydrophilic. The greater hydrophilic character for electropolymerized films thus appears to be associated with biofouling-related performance degradation. Of note, the measured contact angles for each interface was between 45 and 75°. In this manner, all films studied could be described as hydrophilic. Expectations based on hydrophobicity alone are likely not sound given the coulombic interactions of net negatively charged blood plasma proteins and the positively charged electrooxidized films. The favorable selectivity, LOD, and NO permeability of poly-5A1N and poly-phenol modified electrodes in PBS motivate the use of an outer protective membrane—such as Nafion or a fluorinated xerogel—to maintain analytical performance in proteinaceous media.

Conclusions and Outlook

Herein, we studied sensor performance (i.e., NO sensitivity and selectivity) as a function of both monomer identity and depositional protocol. Electrodeposition via CV proved superior to CPA in producing more selective NO sensors. While phenylenediamine monomers are more commonly employed to fabricate NO sensors, both poly-phenol and poly-5A1N modified electrodes proved to be superior with respect to NO sensitivity and selectivity. These analytical merits motivate future study and implementation of poly-phenol and poly-5A1N films for electrochemical NO-sensing platforms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Cyclic voltammograms collected at 2 mm (dia) Pt disk electrodes in 10 mM monomer solutions of (A) *o*-PD, (B) *m*-PD, (C) *p*-PD, (D) phenol, (E) eugenol, and (F) 5A1N in 0.01 M PBS. The pH of the *o*-PD, *m*-PD, *p*-PD, and phenol solutions was 7.4. The eugenol and 5A1N solutions were titrated to pH 13 and 1.0 with NaOH and HCl, respectively. Scan rate was 10 mV s⁻¹.

Figure 2.

Cyclic voltammograms collected during the electrodeposition of 10 mM phenol in 0.01 M PBS (pH 7.4) using a 2 mm (dia) Pt disc electrode, with cycle numbers provided. The dashed line represents the first cycle (cut off for clarity of successive cycles). The scan rate was 10 mV s⁻¹.

Figure 3.

The initial (first) cyclic voltammogram collected during the electropolymerization of phenol from 10 and 100 mM monomer solutions (red and black lines, respectively) in 0.01 M PBS (pH 7.4) as a function of scan rate (10 and 100 mV s^{-1} depicted as dashed and solid lines, respectively).

Figure 4.

Amperograms of the initial 10 s of CPA electro-polymerizations of phenol in 0.01 M PBS (pH 7.4) at +0.6, +0.75, and +0.9 V (solid, dashed, and dotted lines, respectively) in 10 and 100 mM monomer solutions (red and black lines, respectively). Inset: subsequent 30 s of electropolymerization. Curves are baseline corrected from the average current recorded in the last 10 s of electropolymerization (900 s deposition time).

Figure 5.

(A) Retention of NO sensitivity for polymer-modified Pt electrodes in SWF relative to PBS (significance with respect to Pt; $n = 4$). (B) Detection limit ($S/N = 3$) of electrodes in PBS, SWF, and after 1 h of SWF immersion (significance with respect to LOD in PBS; $n = 4$). (C) Static contact angle measurements on polymer-modified planar Pt electrodes (significance with respect to Pt; $n = 4$). * $p < 0.05$; ** $p < 0.01$.

Table 1.

Optimal electropolymerization deposition techniques observed in six self-terminating films and relevant analytical merits for selective nitric oxide detection (n = 4).

Monomer	Technique	Optimized Parameters	LOD ^a (nM)	NO Permeability ^b	NO ₂ ⁻	AA	AP	log <i>k</i> _{NOj} ^c	DA	NH ₄ ⁺	H ₂ O ₂
<i>o</i> -PD	CV	10 mM, 10 mV s ⁻¹	16 ± 9	0.36 ± 0.08	-2.5 ± 0.2	-3.6 ± 0.1	-1.7 ± 0.4	-1.6 ± 0.1	-1.6 ± 0.1	-2.8 ± 0.0	-0.2 ± 0.1
<i>m</i> -PD	CV	100 mM, 100 mV s ⁻¹	90 ± 40	0.13 ± 0.04	-1.8 ± 0.2	-3.5 ± 0.3	-3.7 ± 0.1	-1.9 ± 0.2	-1.9 ± 0.2	-1 ± 1	0.2 ± 0.1
<i>p</i> -PD	CV	10 mM, 10 mV s ⁻¹	11 ± 6	0.50 ± 0.14	-2.7 ± 0.1	-2.8 ± 0.1	-2.7 ± 0.4	-2.6 ± 0.2	-2.6 ± 0.2	-2.9 ± 0.4	-0.5 ± 0.1
Phenol	CPA ^d	100 mM, +600 mV	6 ± 5	1.10 ± 0.29	-4.0 ± 0.1	-3.0 ± 0.2	-3.2 ± 0.2	-2.5 ± 0.1	-2.5 ± 0.1	-3.3 ± 0.2	-2.2 ± 0.1
Eugenol	CV	100 mM, 10 mV s ⁻¹	130 ± 70	0.01 ± 0.00	-3.0 ± 0.4	-2.5 ± 0.1	-2.3 ± 0.5	-1.9 ± 1.0	-1.9 ± 1.0	-2.8 ± 0.3	-1.3 ± 0.2
5A1N	CV	10 mM, 10 mV s ⁻¹	4 ± 3	1.72 ± 0.15	-3.5 ± 0.1	-3.1 ± 0.2	-3.4 ± 0.2	-2.4 ± 0.1	-2.4 ± 0.1	-3.1 ± 0.0	-0.8 ± 0.0
Bare Pt	---	---	10 ± 8	1	-1.2 ± 0.3	0.0 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	-1.4 ± 0.1	-0.4 ± 0.1

^a *S*/*N* = 3.

^b Ratio of film-modified and bare Pt electrode NO sensitivities.

^c Selectivity coefficient against interferent *j* calculated from Eq. 1. Injections of nitrite (NO₂⁻, 1 mM), L-ascorbate (AA, 1 mM), acetaminophen (AP, 250 μM), dopamine (DA, 100 μM), ammonium (NH₄⁺, 1 mM), and hydrogen peroxide (H₂O₂, 1 mM) were made into deoxygenated 0.01 M PBS (pH 7.4; +0.8 V).

^d Not statistically superior to the optimal CV deposition (10 mM, 100 mV s⁻¹).