Reexamination of the Direct Electrochemical Reduction of *S*-Nitrosothiols

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Abstract
We report here on the electrochemical reduction of *S*-nitrosothiol species (RSNO). Nitric oxide (NO) is the reported common product from electrochemically reduced RSNOs at physiological pH. However, studies here at pH 7.4 show that during the reduction of RSNOs (−0.6 V to −0.9 V, vs. Ag/AgCl), no significant amount of NO is detected. Gas analysis suggests RSNO are reduced to nitrous oxide (N_2O) at pH 7.4 and can only be converted back to NO at more oxidizing voltages. Interestingly, at pH 4.0, a direct one-electron reduction of RSNOs appears to occur and generates significant amounts of NO from RSNO species.

Keywords: *S*-Nitrosothiols, Nitric oxide, Nitrous oxide, Electrochemical reduction

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Dedicated to Professor Erkang Wang on the Occasion of His 80th Birthday

1 Introduction

Since nitric oxide (NO) was first discovered as the elusive endothelial-derived relaxing factor (EDRF) in 1987, this radical gas species is well recognized for playing critical roles in platelet inhibition, vasodilation, and wound healing [1–3]. However, NO does not exist to any significant degree in blood due to its rapid reaction with oxyhemoglobin to form methemoglobin and nitrate [3]. *S*-Nitrosothiols (RSNOs) are potent donors of NO and can release localized transient levels of NO under physiological conditions via various decomposition pathways (thermal, photolysis, catalytic metal ion reduction, etc.) [4]. Endogenous RSNOs are formed in vivo from the reaction between thiols and reactive oxidized intermediates of NO (e.g., N_2O_3). Approximately 20% of NO produced by nitric oxide synthase (NOS) in the vasculature is scavenged by hemoglobin, and the remainder is oxidized to various species (NO_2^-, etc.), including those that can react with thiols, either catalytically or non-catalytically, to form low concentrations of RSNOs. Chemically, *S*-nitrosothiols are thioesters of nitrite with the nitroso moieties covalently bound to sulfhydryl groups. The S–NO group decomposes rapidly upon a one-electron reduction in vivo, and therefore provides a selective means to release NO from RSNOs in physiological systems. This reduction reaction can be catalyzed by Cu(I/II) or organoselenium species, such as proteins that contain Cu(I/II) centers or selenocysteine groups [5].

Previous research has demonstrated that physiological levels of NO ($0.5–4\times10^{-10}$ mol cm^{-2}·min^{-1}) can be released from polymeric materials by incorporating NO donors (diazeniumdiolates or *S*-nitrosothiols) within such matrices [6–8]. In particular, photo-switchable NO releasing films made of RSNO modified fumed silica particles doped into silicone rubber polymers function by utilizing light to liberate NO from these materials [9]. The kinetics of NO release from such materials can be modulated by changing the polymer matrix or the structure of the NO donor. Other methods of preparing NO-releasing polymers consisting of small-molecule RSNOs donors, such as *S*-nitroso-*N*-acetyl-L-cysteine (SNAC) and *S*-nitrosoglutathione (GSNO), dispersed in hydrogels have been reported by the Oliveira [10] and Schoenfisch [11] groups. The NO delivery for these systems was accomplished by thermal, photochemical, or catalyst (copper ion, Cu^+) activation.

Based on the known one-electron reduction chemical reactions that can liberate NO from RSNOs in solution, it was thought that electrochemical reduction of RSNOs could provide a potential alternate approach to realize quantitative NO release in a controlled manner. In fact, it has been reported previously that RSNOs can undergo a one-electron reduction and release NO on glassy carbon or hanging mercury drop electrodes at physiologi-

cal pH [12–14]. Controlled potential electrolysis suggested that NO was released at cathodic potentials [15]. However, in these prior studies, the conclusion that NO was a product upon electrochemical reduction of RSNOs was based solely on cyclic voltammetry in which following reduction, sweeping to more positive working electrode potentials yielded an oxidation peak that corresponded to the potential where NO is typically oxidized. This previous research inspired us to consider a new method to create NO releasing materials. By tuning the applied reduction potential via an electrode within a matrix with embedded RSNOs, a novel approach for creating voltage-triggered NO release materials could ultimately be developed. This type of control would allow fundamental studies to define necessary NO fluxes required to achieve specific therapeutic effects. Moreover, the use of a voltage trigger to initiate release provides a method of temporal control of the NO flux.

Toward this goal, preliminary studies were conducted to verify that both physiological (*S*-nitrosoglutathione (GSNO), *S*-nitrosocysteine (CysNO)) and synthetic RSNOs (*S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP), *S*-nitroso-*N*-acetyl-L-cysteine (SNAC)) can be electrochemically reduced. However, during these studies no evidence of NO gas production could be found at pH 7.4, indicating that NO is not necessarily the predominant reductive product, or a multiple-step electrochemical reaction, rather than a simple one-electron transfer, is involved in the electrolysis. To better understand the reaction and provide critical information to assess the potential application of using the electrochemistry of RSNOs in creating new electrochemical NO releasing devices, it is important to characterize the electrochemical decomposition path and sort out the actual reductive products. Hence, results from detailed solution and gas phase analysis via both electrochemical and spectroscopic methods are presented herein regarding the electrochemical reduction of *S*-nitrosothiols.

2 Experimental

2.1 Materials

Glutathione (GSH), cysteine (Cys), *N*-acetyl-L-cysteine (NAC), *S*-nitroso-*N*-acetyl-DL- penicillamine (SNAP), ethylenediamine tetraacetic acid (EDTA), *N*-(1-naphthyl)ethylene diamine dihydrochloride (NED) obtained from Sigma-Aldrich (Milwaukee, WI) and sulfanilamide from Acros (Morris Plains, NJ) were used without further purification unless otherwise noted. A standard $NO_{(g)}$ stock solution was prepared from deoxygenated acidified nitrite solution. The generated gas was bubbled through 20% (wt%) NaOH solution to remove any interference nitrogen dioxide species and then collected in deoxygenated DI water for 30 min. Via use of a nitric oxide analyzer (NOA) (Seivers 280, Boulder, CO), the concentration of NO in a saturated solution was determined to be 1.9 mM at 25 °C and $P_{NO} = 1$ atm. *S*-Nitrosoglutathione (GSNO), *S*-nitrosocysteine (CysNO), and *S*-nitroso-*N*-acetyl-L-cysteine (SNAC) were synthesized by nitrosating the sulfhydryl groups of the corresponding thiols (GSH, CySH, and NAC) in acidified nitrite solution, as reported in the literature [16]. The formation of the *S*-nitroso group was confirmed by a characteristic absorption at 335 nm via UV-Vis spectrophotometry. Phosphate buffered saline (PBS) and all other solutions were prepared with 18.2 MΩ cm^{-1} DI water from a Milli-Q system (Millipore Corp., Billerica, MA).

2.2 Electrochemical Reduction of RSNO

The RSNOs stock solutions (5 mM) were directly injected into a working buffer solution (PBS, 10 mM, pH 7.4) containing 0.1 mM EDTA (necessary to suppress metal ion impurities and prevent catalytic RSNO decomposition, especially from copper ions) to obtain the desired concentrations. All RSNO measurements were carried out in amber cells to avoid photolytic decay. A potentiostat (CHI 800B, Austin, TX) was used to examine the electrochemistry of RSNOs via cyclic voltammetry and chronoamperometry techniques. Preliminary cyclic voltammetry experiments of four different RSNOs (GSNO, CysNO, SNAC, SNAP) were tested on both polycrystalline gold and glassy carbon electrode surfaces (3 mm in diameter, CHI, Austin, TX) in 1 mM RSNO solutions prepared in deoxygenated PBS. GSNO was then selected as the model molecule for all other electrochemical reduction reactions studies due to its good stability (up to a few hours) and physiological abundance. Typically, a solution of 2 mM GSNO in stirred PBS was reduced at a potential of −0.8 V (vs. Ag/AgCl) on a Au mesh or glassy carbon electrode for 1 h, using a platinum coil behind a glass frit as the counter electrode. Before all electrochemical reactions, the cell was purged with either nitrogen or argon to prevent the oxidation of end products from any dissolved and headspace oxygen present.

2.3 Detection of NO

To assess whether NO is liberated during the electrochemical reduction of RSNOs, the reaction solution was constantly purged with $N_{2(g)}$ and the gas phase stream was monitored by a highly sensitive and selective chemiluminescence nitric oxide analyzer (NOA). An amber combination echem-NOA cell (10 mL) was specially designed to conduct such combined electrochemical reduction and NO monitoring experiments.

2.4 Detection of NO_2^-

The colorimetric Griess assay was conducted to measure nitrite (NO_2^-) produced from NO after RSNO reduction. A mixture of 15 mM sulfanilamide and 0.23 mM NED in 0.75 M HCl was prepared as the Griess reagent. Prior to sample measurement, the response of the Griess reagent towards a series of standard nitrite solutions was moni-

tored by a UV-Vis spectrophotometer (Lambda 35, Perkin-Elmer, MA). The strong absorbance at a λ_{max} of 540 nm was plotted against NO_2^- concentrations to obtain the calibration curve. A 500 μL aliquot of the RSNO reduction solution was injected into a cuvette with 500 μL of Griess reagent and kept on the bench for 10 min to fully mix and react. The recorded absorbance at 540 nm was then fitted into the calibration curve to calculate the NO_2^- concentration.

2.5 Detection of Ammonia (NH_3)

Potentiometric measurement of ammonia was employed to evaluate whether NH_3 was one of the electrochemical reduction products of RSNOs. A high performance NH_3 gas sensor (Thermo Scientific, Beverly, MA) was used to measure the concentration of dissolved ammonia gas in GSNO solutions after electrochemical reduction. The sensor was mounted with Teflon gas-permeable membrane and preconditioned in 0.1 M NH_4Cl solutions overnight and then in 15 μM NH_4Cl for 30 min prior to use. Small aliquots of 1 mM NH_4Cl solution were first added into a 0.2 M NaOH solution to calibrate the response to NH_3 over a broad (10^{-6} to 10^{-2} M) range. A 2 mL sample solution of electrochemically reduced GSNO (reduction for 30 min at gold mesh electrode) was then introduced to a fresh 2 mL NaOH solution with a 1:1 dilution for measurement.

2.6 Detection of NO^-/N_2O

Gas phase FT-IR spectroscopy (Spectrum BX, Perkin-Elmer, MA) was utilized to verify the existence of N_2O that would be produced from NO^- after GSNO decomposition. An amber electrochemical cell (50 mL) was aligned with a CaF_2 windowed (Pike Tech., Madison, WI) IR gas cell to collect the headspace gas from a GSNO solution after electrochemical reduction for 60 min at gold mesh electrode. Because of the high reactivity of N_2O with O_2, the GSNO solution was first purged with argon for 30 min prior to reaction and the IR gas cell was evacuated by vacuum before collecting the reductive headspace gas.

3 Results and Discussion

3.1 Preliminary Studies on the Electrochemical Reduction of RSNOs

For preliminary studies, cyclic voltammetric experiments were carried out for both physiological (GSNO, CysNO) and synthetic (SNAC, SNAP) RSNO species at polycrystalline gold and glassy carbon electrodes. As shown in Figure 1, the various RSNOs at 1 mM concentrations can be electrochemically reduced, exhibiting an irreversible cathodic wave at a potential of −0.8 V (vs. Ag/AgCl) on gold electrode surface at pH 7.4. The reduction of 1 mM $NO_{(g)}$ is overlaid as trace b in Figure 1, showing a different

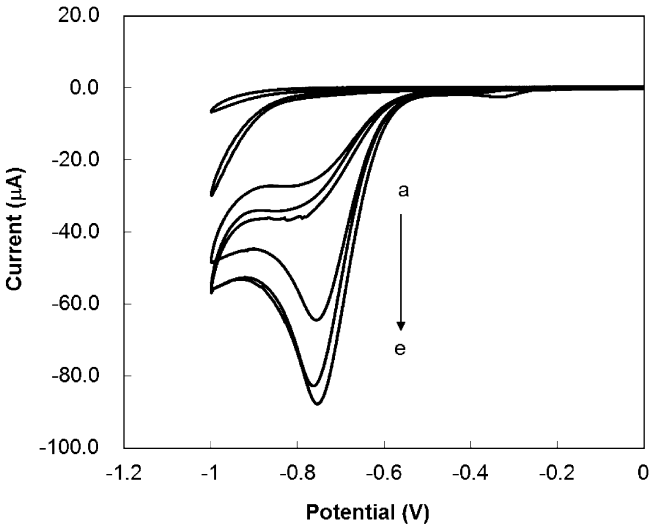

Fig. 1. Cyclic voltammograms of 1 mM NO and RSNOs in 10 mM PBS on a gold electrode at a scan rate of 0.05 V/s (vs. Ag/AgCl), a) blank PBS, b) NO, c) SNAC, d) GSNO, e) CysNO.

voltammogram pattern where the cathodic current starts increasing from −0.8 V and does not yet give a clear reductive peak even at −1.0 V. Therefore, it can be concluded that the observed cathodic peaks for the RSNO solutions are not due to the reduction of dissolved NO originating from RSNO self-decomposition (thermal, photo, etc.). Rather the reduction peak must be due to direct electrochemical reduction of the *S*-nitroso bond.

The reduction peaks for RSNOs appear as totally irreversible waves in which the observed peak potential (E_p) is a function of scan rate, shifting in a negative direction with the increase in scan rate (v) (see Figure 2). This irreversible behavior suggests that the *S*-nitroso bond of GSNO has been electrochemically cleaved and can no longer be oxidized back on the electrode surface. Furthermore, plotting the peak current (i_p) versus the square root of scan rate ($v^{1/2}$) shows that i_p is proportional to $v^{1/2}$, indicating a diffusion controlled process (Figure 2 Inset) [17].

Very similar data to that shown in Figures 1 and 2, are obtained when using a glassy carbon electrode as the working electrode for reduction of RSNOs (see Supporting Information file, Figure S1). This suggests that the electrochemical reduction of RSNOs occurs by similar pathways on both types of electrodes.

3.2 NO Produced from RSNOs

3.2.1 Chemiluminescence Detection

To further investigate the electrochemical reaction, a highly sensitive chemiluminescence nitric oxide analyzer (NOA) was used to assess whether NO is in fact liberated upon the electrochemical reduction of the *S*-nitroso bond of RSNOs. The generated NO can undergo a rapid reaction in the gas phase with ozone, yielding ni-

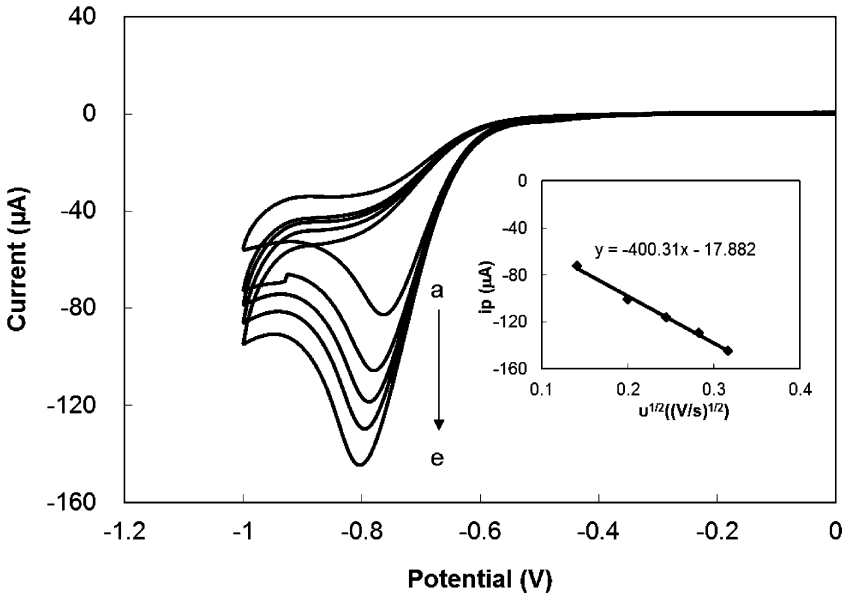

Fig. 2. Cyclic voltammograms of 1 mM GSNO in 10 mM PBS on gold electrode at different scan rates (V/s) a to e: 0.02, 0.04, 0.06, 0.08, 0.10 V/s; Inset: i_p versus $v^{1/2}$ fit into a linear regression.

trogen dioxide (NO_2) in an excited state. As the excited electron returns to the ground state, a photon is emitted and detected as chemiluminescence. This highly specific, sensitive and reliable method is considered to be the gold standard for trace level NO detection [18].

Surprisingly, when a cathodic potential of −0.8 V (vs. Ag/AgCl) was applied using a large area gold mesh working electrode (2.5 cm × 3.5 cm, 100 mesh) and the solution (10 mM PBS, pH 7.4) was purged with nitrogen continuously (into NOA), no significant NO increase is observed via the NOA analyzer (see Figure 3). Instead, the initial NO flux derived from the auto-decomposition of GSNO (about 4×10^{-12} mol/s) actually decreases when the cathodic potential was applied (see Figure 3). This is likely from the electrochemical reduction of NO at this potential, as observed from trace b in Figure 1. This result indicates that NO is not likely the predominant product of the electrochemical RSNO reduction reaction at pH 7.4, or a multiple-step electrochemical reaction rather than a discrete one-electron transfer is involved in the reduction of RSNOs.

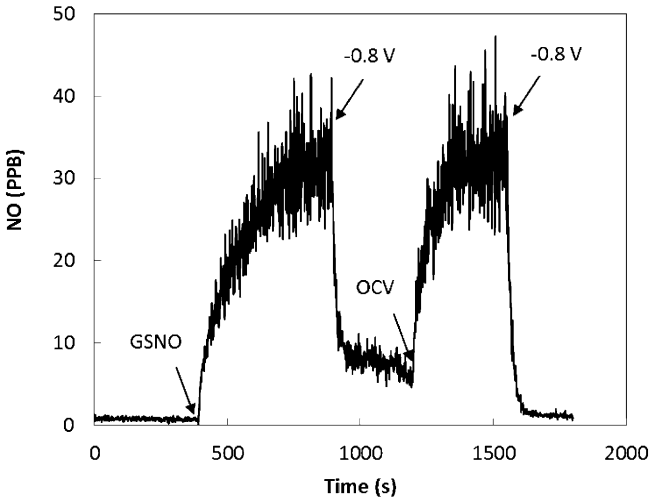

Fig. 3. NO profile of 10 μM GSNO during electrochemical reduction at −0.8 V (vs. Ag/AgCl) and open-circuit voltage (OCV) on a gold mesh electrode surface.

3.2.2 Griess Assay for Nitrite Detection

Besides the chemiluminescence method to detect NO, a spectrophotometric Griess assay was employed to measure nitrite ion formed by the possible oxidation of NO with oxygen. In brief, under acidic conditions, sulfanilamide reacts with NO_2^- to form a diazonium cation that further reacts with *N*-(1-naphthyl)ethylenediamine, producing a diazo molecule characterized by a strong absorbance at 540 nm.

This type of spectroscopic method is highly sensitive and can yield a detection limit as low as 1 μM, thus it is an ideal tool to monitor the level of any nitrite ions formed in the GSNO solutions as a function of the electrochemical reduction time of RSNOs. Unfortunately, when a sample of 1 mM GSNO solution was electrolyzed (at −0.8 V for 60 min at gold mesh electrode) and then analyzed by this method, there was very little absorbance at 540 nm (<1 μM equivalent nitrite, data not shown), further suggesting that no significant amount of NO was produced from the electrochemical reduction of GSNO at pH 7.4.

3.3 Possible Reductive Products of RSNOs

Since all previous reports state that NO was the predominant reductive product from the electrochemical reduc-

tion of RSNOs at physiological pH [12–14], the data shown in Figure 3 are rather surprising. Although most decomposition pathways of RSNOs mainly lead to the homolytic cleavage of the S–NO bond and formation of the NO free radical, these pathways may not be exclusive because the chemistry of these compounds is complex. Indeed, there are several possible candidates besides NO, such as ammonia gas, nitrosonium ions, and nitrosyl ions, that may be produced.

3.3.1 Ammonia (NH_3)

Ammonia has been reported as one of the end products of the redox reaction between RSNO and high levels of a given thiol [19]. Therefore, we investigated whether direct electron transfer via an electrochemical method can also lead to the formation of ammonia in the RSNO solution that undergoes electrolysis at −0.8 V.

Severinghaus-type NH_3 electrodes are based on a gas-permeable membrane that separates the sample from an internal solution in which a glass electrode monitors the pH [20]. These devices are used to reliably measure dissolved ammonia, and ammonium ions by adding base to convert ammonium to ammonia gas. Such electrodes have been approved by the United States Environmental Protection Agency (EPA) and are also described as a standard method for ammonia detection [21].

Indeed, the ammonia electrode exhibits a fast response and wide dynamic range from 10^{-6} M to 10^{-2} M towards ammonia (Figure 4a), providing an excellent analytical tool to measure ammonia produced due to the electrochemical reduction of GSNO at a large area gold mesh electrode. However, as shown in Figure 4b, after reduction of 2 mM GSNO for 30 min with stirring at pH 7.4, there is no immediate ammonia sensor response upon the addition of a sample of the GSNO solutions, except for a slow drift that is mostly due to accumulation of dissolved ambient ammonia. Moreover, there was no significant difference between samples that was blank PBS and the one underwent 30 min of cathodic reduction, indicating no appreciable amount of ammonia is produced from the electrochemistry of GSNO.

3.3.2 Nitrosyl Ion (NO^-)

As previously reported, RSNOs can also react with thiols and produce NO^-, which under appropriate conditions can yield either nitrous oxide (N_2O) or peroxynitrite ($OONO^-$) [22]. More specifically, NO^- as an active intermediate will undergo a fast dimerization reaction to form N_2O at a rate of $8\times10^6\ M^{-1}s^{-1}$ under anaerobic conditions, or the reaction with oxygen to form $OONO^-$ under aerobic condition [23]. The existence of NO^- is inferred by the detection of N_2O since it has an advantage of being infrared active with simple asymmetric stretch absorptions in IR spectroscopy at 2210–2230 cm^{-1} that is different from the linear stretching at 1780–1960 cm^{-1} for NO [24].

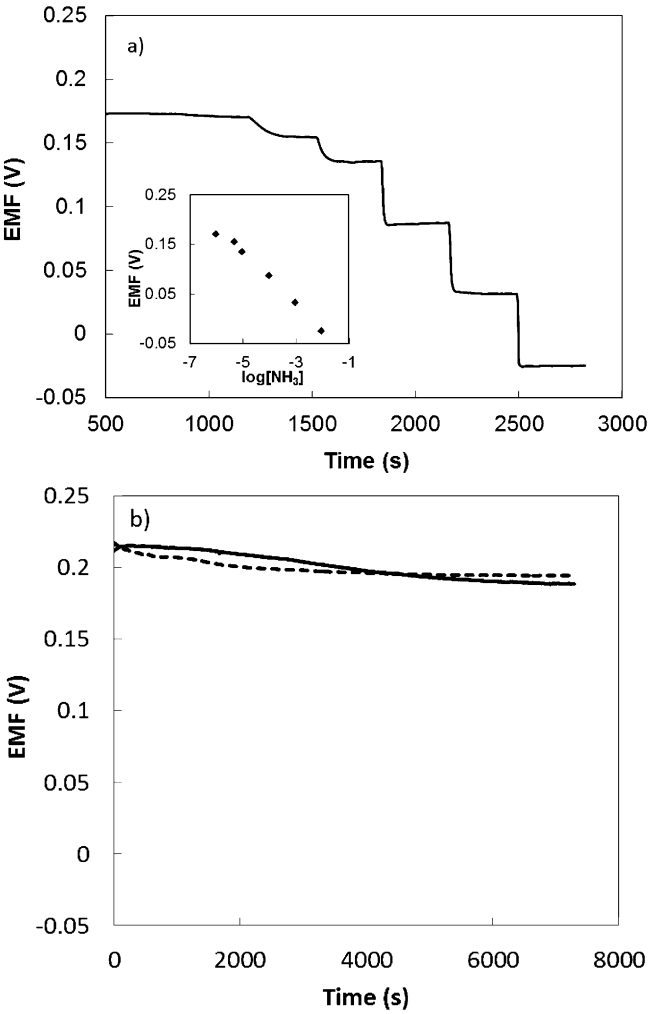

Fig. 4. a) Response and calibration (inset) curves towards concentrations (10^{-6} to 10^{-2} M) of NH_3, exhibit a sub-Nerstian response with a slope of −53.7 mV/decade. b) EMF response with the addition of GSNO solutions (reduced at −0.8 V for 30 min, solid line) and the control blank PBS (dashed line).

Therefore, anaerobic headspace gas analysis was conducted to elucidate whether NO^-/N_2O is a reduction product from the electrochemical reaction of RSNOs at pH 7.4. Beforehand, 2 mM solution of saturated NO in 100 mM PBS was reduced at a potential of −0.8 V (vs. Ag/AgCl) on the Au mesh electrode for 1 h. The gas phase FT-IR spectrum of the headspace gas shown in Figure 5 confirms the presence of N_2O at wavenumbers 2211 cm^{-1} and 2235 cm^{-1}, strongly indicating that N_2O is a reductive product of NO (stretching bands at 1790 and 1810 cm^{-1}) at −0.8 V on a Au surface. Another identifiable absorbance band at a lower wavenumber of 1630 cm^{-1} is probably due to trace amounts of NO_2 present. Most importantly, when a solution of 2 mM GSNO in 100 mM PBS (pH 7.4) undergoes the same electrochemical reduction on either Au or glassy carbon electrodes, bands at 2211 cm^{-1} and 2235 cm^{-1} appear to prove that N_2O is the major product of the electrochemical RSNO reduction (see Figure 6).

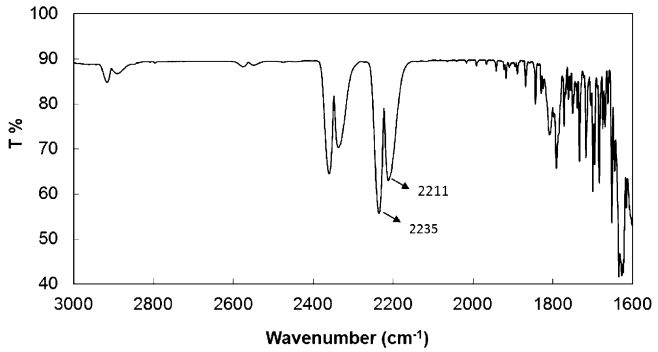

Fig. 5. FT-IR spectrum of headspace gas from deoxygenated 2 mM NO in 100 mM PBS (pH 7.4), after reduction at −0.8 V (vs. Ag/AgCl) on Au mesh electrode for 1 h.

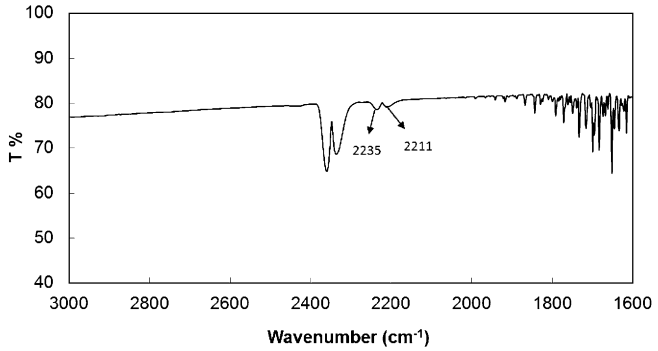

Fig. 6. FT-IR spectrum of headspace gas from deoxygenated 2 mM GSNO in 100 mM PBS (pH 7.4), after reduction at −0.8 V (vs. Ag/AgCl) on Au mesh electrode for 1 h.

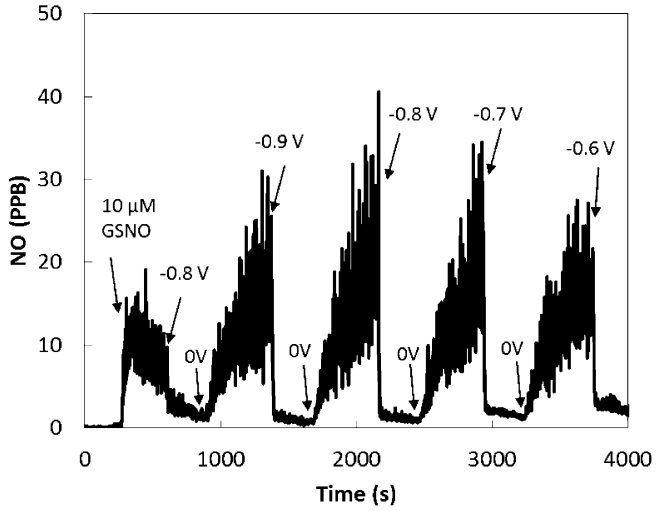

Fig. 7. Nitric oxide profile of 10 µM GSNO in 100 mM PBS (pH 7.4), first in absence of any potential application, and then after different potentials are applied to gold mesh electrode.

Moreover, a series of pulsed reduction potentials (between −0.6 V to −0.9 V) were applied to a gold mesh electrode in the presence of 100 µM GSNO in PBS solution at pH 7.4. The NOA results shown in Figure 7 exhibit an interesting "up-and-down" pattern when the applied voltage is switched between anodic (0 V) and cathodic potentials. The reducing potentials result in an immediate NO decrease, and by switching back to the oxidizing potential (0 V) leads to an increase of NO concentration. These results suggest that at pH 7.4 under reduction potentials between −0.6 V to −0.9 V, any NO that results from GSNO is further reduced into another reductive product, which can then be oxidized back to NO at a more anodic potential.

Indeed, two possible reductive pathways may occur upon the electrochemical reduction of RSNOs (see Scheme 1) at pH 7.4. A one-electron transfer reaction (to form transient NO, reaction 1) followed by an immediate second electron transfer (Reaction 2) to create N_2O (reaction 4) is one possibility. Another option is a direct one-electron transfer process to two RSNOs molecules that will also produce N_2O via a nitrosyl intermediate (Reactions 3–4).

$$RSNO + e^- \rightarrow NO^{\bullet} + RS^- \quad (1)$$

$$NO^{\bullet} + e^- \rightarrow NO^- \quad (2)$$

$$RSNO + e^- \rightarrow NO^- + RS^{\bullet} \quad (3)$$

$$2NO^- + H_2O \rightarrow N_2O + OH^- \quad (4)$$

Scheme 1. Proposed reactions for the electrochemical reduction of RSNOs.

To calculate the number of electrons transferred in the reactions, UV-Vis spectroscopy was utilized to quantitatively monitor the amount of RSNOs remaining in solution after bulk electrolysis of GSNO at −0.8 V. The characteristic absorbance of *S*-NO at 335 nm was detected before and after GSNO was reduced. With a molar extinction coefficient of 0.85 $mM^{-1}cm^{-1}$, the amounts of consumed GSNO after one hour of reduction was 5.94×10^{-6} mol. At the same time, the total charge passed through the electrochemical reaction was 1.83 coulombs, which was produced by 1.90×10^{-5} mol of electrons. Based on these results, an estimated number of electrons transferred is 3.2 per reaction. Clearly, this does not agree with either of the one-electron or two-electron transfer reactions suggested above. This could be explained by the many possible side reactions that can occur at the same time and would result in more than the expected number of electrons. For example, one possibility is the reaction between HNO (produced by protonation of NO^-) and free thiols in solution, to further reduce HNO and produce hydroxylamine (NH_2OH) and a disulfide. A two electron reduction of the disulfide could occur at the reductive potentials applied in the above experiments, increasing the number of electrons observed under coulometric conditions. In theory, hydroxylamine can also be reduced, but the product of this electrochemical reduction reaction is usually ammonia [25], and hence there is no evidence that hydroxylamine, if formed, is also being

reduced electrochemically at the potentials applied. In contrast, it is known that alkyl disulfides can be reduced in the range from −0.8 V to −1.0 V on gold electrode [26], and hence the presence of significant levels of these species could explain the coulometry results.

3.4 pH Effect on Electrochemical Reduction of RSNO

All previous studies reported in the literature, as well as those described above, were carried out at pH 7.4, given that endogenous *S*-nitrosoglutathione would exist at this physiological pH. However, preliminary studies regarding the effect of pH on the electrochemical reductive pathway of RSNOs were also carried out at pH 4.0 and 10. Figure 8 shows the cyclic voltammograms of GSNO on gold disk electrode in pH 4.0 PBS at different scan rates. The negative shift at higher scan rate suggests an irreversible reduction of the *S*-nitroso bond. The cathodic peak potentials are similar to those shown in Figures 1 and 2, with only 50 mV more positive peak voltage occurring at pH 4.0 compared to pH 7.4.

Most interesting, however, is that in a solution of 10 μM GSNO in 100 mM PBS at pH 4.0, when a cathodic potential of −0.8 V (vs. Ag/AgCl) is applied using a large area gold mesh working electrode (2.5 cm × 3.5 cm, 100 mesh) and the solution is purged with nitrogen continuously (into NOA), a significant NO burst is clearly observed via the NOA analyzer (see Figure 9). Using a 5 min interval between application of −0.8 V and 0 V to the gold mesh electrode, an "on" and "off" pattern of NO release is clearly observed. More importantly, by switching the potential on and off repeatedly, 97 % of the total GSNO is converted into NO after 5 such cycles (Figure 9).

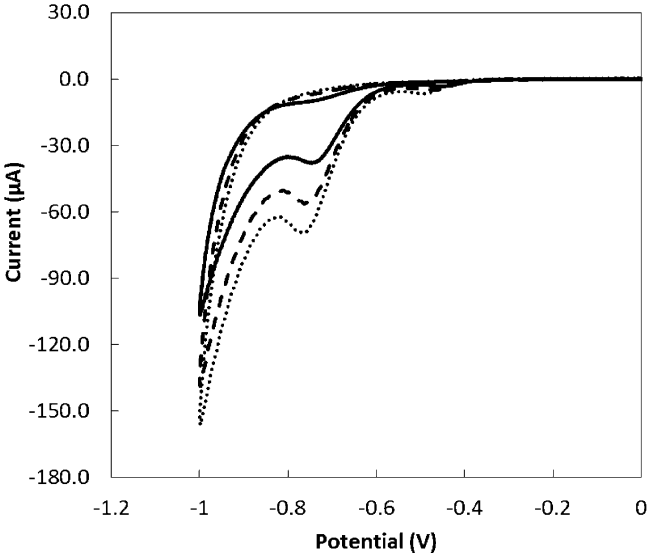

Fig. 8. Cyclic voltammograms of 1 mM GSNO in 100 mM PBS (pH 4.0) on gold electrode at different scan rates (V/s): 0.02 (solid line), 0.05 (dashed line), 0.08 (dotted line) V/s.

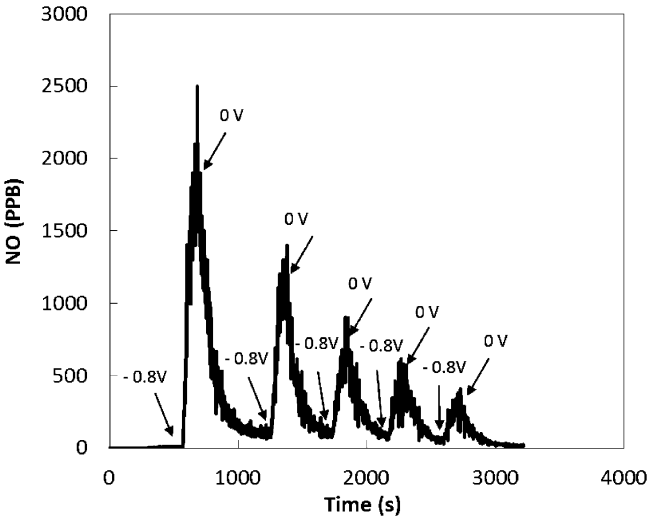

Fig. 9. Nitric oxide profile of 10 μM GSNO in 100 mM PBS at pH 4.0, first in absence of any potential application, and then after cathodic potentials of −0.8 V (vs. Ag/AgCl) are applied to gold mesh electrode.

The results for NO release experiments at pH 4.0 are clearly very different than observed at pH 7.4. To assess whether this difference carries over to other RSNO species, the electrochemical reduction of synthetic SNAP at pH 4.0 was also examined. Similar quantitative NO release is observed at −0.8 V at pH 4.0 for this RSNO species (data not shown). In addition, acetate buffer was also employed to eliminate any possible buffer effect, and the same reductive NO release pattern is observed. Overall, the reduction pathway of RSNOs at low pH most likely involve a proton that helps favor the direct one electron reaction, with protonation of the thiolate helping to drive the reaction to the right in accordance with reaction 5:

$$RSNO + H^+ + e^- \rightarrow NO^{\bullet} + RSH \quad (5)$$

At more basic pH conditions, pH 10, the electrochemical reduction behavior is quite similar to that observed at pH 7.4 (except for a higher NO baseline due to poor GSNO stability at high pH), with no significant amounts of NO produced when cathodic potentials are applied onto gold mesh electrode (NO profile provided in Supporting Information file, Figure S2).

4 Conclusions

Contrary to previous literature [12–14], the results reported herein suggest that during the electrochemical reduction of RSNOs at voltages between −0.6 V to −0.9 V at pH 7.4, $NO_{(g)}$ is not the predominant product. Instead, the RSNO species are reduced to $N_2O_{(g)}$ and can only be converted back to NO by switching to a more oxidizing potential. Two possible reductive pathways may occur upon the electrochemical reduction of RSNOs to explain the production of N_2O rather than NO. Further experi-

ments aimed at accounting for all the possible electro-reduction reactions that lead to an unexpected high number of electrons from coulometric measurements at pH 7.4 are needed before final conclusions can be drawn. However, at pH 4.0, quantitative NO release from RSNOs at a gold working electrode does indeed take place at a cathodic potential of −0.8 V. Additional experiments aimed at examining other pH values between pH 4.0 and 7.4 with various RSNO species still need to be conducted in order to better understand the role of proton activity on the electroreduction of RSNOs. Nonetheless, based on preliminary results obtain here, it seems possible to use a reservoir of RSNOs at low pH to create biomedical devices (e.g., intravascular catheters) in which electrochemically modulated release of NO can be achieved to prevent clotting and infection.

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