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Direct electrochemical sensing of hydrogen sulfide without sulfur poisoning

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Abstract

An electrochemical method capable of direct, real-time detection of hydrogen sulfide was developed using triple pulse amperometry (TPA) to mitigate sulfur poisoning and its related passivation of the working electrode surface. Through repeated cycles of discrete potential pulses, the electrooxidation of surface-adsorbed elemental sulfur to water-soluble sulfate ions was exploited to regenerate the glassy carbon electrode surface and maintain consistent sensor performance. Amperometric measurements and X-ray photoelectron spectroscopy surface analysis demonstrated that the TPA sensors provided enhanced analytical performance via decreased sulfur accumulation relative to low-potential (+0.7 V) constant potential amperometry. Sensors operated under optimized TPA parameters retained high sensitivity (57.4 ± 13.0 nA/ μ M), a wide linear dynamic range (150 nM - 15 μ M), fast response times (<10 s), and a sub-micromolar detection limit (<100 nM) upon consecutive calibration cycles. The sensitivity and response time achieved were comparable to or better than current electrochemical sensors. Moreover, the simplicity of the method eliminates the need for external redox mediators or semi-permeable membranes.

Graphical Abstract

Keywords

Hydrogen sulfide; electrochemical oxidation; triple pulse amperometry; glassy carbon; sensors

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Supporting Information

Cyclic voltammograms at pH 7.4 and passivation tests at various working potentials on GC and Pt, additional sulfur XPS spectra after applying different working potentials and altering the number of calibrations, sensitivity retention while varying pulse ratios and the cleaning pulse potential, and calibration curve in proteinaceous media.

Until recently, hydrogen sulfide (H₂S) was known only as a noxious gas and weak diprotic acid.^{1,2} With a reported pK_a between 6.7 and 7.1, hydrogen sulfide exists as both H₂S and hydrosulfide (HS⁻) in neutral, aqueous solutions.^{2,3} A product of decaying organic matter with acute toxicity at low concentrations (>500 ppm), H₂S has long been deemed an environmental and occupational hazard.^{4,5} Much initial work on the biological activity of H₂S has thus focused on its harmful side effects, and in particular, its ability to bind to and inhibit cytochrome *c* oxidase within mitochondria.⁶ Hydrogen sulfide is now included in the family of endogenously-produced gaseous signaling molecules known as gasotransmitters, which includes nitric oxide (NO) and carbon monoxide (CO).⁷ Primarily biosynthesized through the enzymatic metabolism of cysteine, H₂S plays integral roles in a variety of biological processes, including the cardiovascular, endocrine, gastrointestinal, and nervous systems.^{7–10} Indeed, H₂S has been implicated in the control of ion channels (e.g., K_{ATP} channels), protein modifications (e.g., sulphydration of kinases), metabolic pathways (e.g., inhibition of cytochrome *c* oxidase), radical scavenging, and the regulation of transcription factors.^{8,11,12} Similar to other gasotransmitters, H₂S influences anti- and pro-inflammatory responses, vasodilation, angiogenesis, cardioprotection, anti-tumor processes, and neurological diseases and disorders.^{13–18} Hydrogen sulfide-related research has thus evolved to include therapeutic donors.^{19,20} The ability to accurately detect and measure both endogenous and exogenous H₂S remains a significant challenge to further understanding the physiology and pharmacology of this molecule.

Colorimetric reactions, such as iodometric titration and the methylene blue assay, represent the oldest and most widely used methods for H₂S detection and quantification.^{5,21} While simple and quick, these methods suffer from inadequate detection ranges for the suggested physiological concentration, low sensitivity, and lack of specificity for H₂S, making them impractical for most biological measurements without significant sample preparation.^{5,21–23} Fluorescent probes have also been developed to quantify sulfide in living cells. These probes eliminate problems with non-specificity.²⁴ However, fluorophores often require long incubation times, provide only temporally discrete concentration information, and are not capable of detecting H₂S at low concentrations due to tissue autofluorescence.²⁵

Potential H₂S concentration fluctuations in biological systems warranted the development and improvement of real-time detection methods to more accurately assess the physiologically-relevant concentration range.^{6,8} Electrochemical methodologies are best suited for real-time detection as they allow for continuous and sensitive monitoring of redox active species in a simple and cost-effective manner. The byproducts of H₂S oxidation vary depending on the applied potential.²⁶ Sulfur oxides are formed at high potentials, while at low potentials (<+0.2V), the main byproduct is elemental sulfur. Oxidation of H₂S at low potentials results in the production of insoluble elemental sulfur as follows^{26–28}:

Regardless of the working electrode composition, the deposited elemental sulfur passivates the electrode surface decreasing analytical sensitivity.^{28–32} Oxidation of H₂S in contact with the surface of the sulfide layer forms polysulfides, increasing the insulating layer.³³ Any potential for continuous measurement is impeded by the attenuating response to H₂S over time. Jeroschewski et al. previously reported on a sensor capable of avoiding electrode passivation by using an alkaline redox mediator solution (i.e., ferrocyanide) contained between a gas permeable membrane and the working electrode.^{30,34} This redox mediator-based sensor is currently the most widely-used method for real-time electrochemical monitoring of dissolved hydrogen sulfide. However, sensor miniaturization is challenging due to the external redox mediator solution/coating. Current sensors are limited to diameters 100 μm (World Precision Instruments; Sarasota, FL), while uncoated carbon fiber electrodes are capable of <10 μm . Furthermore, redox mediator layers impede the application of an environment-specific surface modifications (i.e., anti-fouling coatings) needed for in vivo measurements.^{6,35}

Prior to 2010, most reports suggested that biological H₂S concentrations spanned 1–100 μM .^{8,36} More recent studies contend that significantly lower concentrations of H₂S (i.e., 100s of nM) mediate biological activity.^{20,37,38} These literature inconsistencies have spawned a debate regarding what the true physiological levels are, and the appropriate therapeutic window for potential H₂S donor delivery. Presently, detection methods alter H₂S through a chemical reaction or modify the proton dissociation equilibrium by manipulating the solvent's pH prior to quantification. Thus, improved analytical methods capable of accurate quantification of H₂S dissolved in aqueous solutions without changing its physiological form are clearly warranted.

In this manuscript, we report an electrochemical approach that directly oxidizes hydrogen sulfide and avoids sulfur poisoning without the use of an external redox mediator, pH manipulation, or permselective membrane. While pulsed electrochemical detection (PED) has been employed previously to selectively detect sulfur-containing compounds on noble metal electrodes for chromatographic purposes,^{32,39–41} we make use of triple pulse amperometry (TPA), a subcategory of PED, to provide discrete cleaning and measuring pulses to recondition the electrode surface. In this manner, a novel pulse scheme is performed using unmodified, nonmetal electrodes at physiological pH. The effectiveness of the cleaning step was evaluated using both electrochemistry and X-ray photoelectron spectroscopy (XPS) to determine the pulse parameters that promote optimal H₂S sensitivity and sensor performance.

Experimental

Materials and instrumentation.

Sodium sulfide nonahydrate (Na₂S·9H₂O), ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA), hydrochloric acid (HCl), sodium nitrite, L-ascorbic acid, acetaminophen,

L-cysteine, L-glutathione, and fetal bovine serum (FBS) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium sulfate, ammonium hydroxide, hydrogen peroxide (30 wt%), sulfuric acid, and sodium hydroxide (NaOH) were purchased from Fisher Scientific (Hampton, NH). Potassium nitrate (KNO_3) was purchased from Mallinckrodt Pharmaceuticals (St. Louis, MO). Nitric oxide (99.5%) and carbon monoxide (99.3%) gases were purchased from Airgas National Welders (Durham, NC). All solutions were prepared with distilled water purified to a resistivity of $18.2 \text{ M}\Omega\cdot\text{cm}$ and total organic content of 6 ppb using a Millipore Milli-Q UV Gradient A10 water purification system (Bedford, MA). All other chemicals were reagent grade and used as received.

Electrochemical measurements were performed using a CH Instruments 1030A eight-channel potentiostat (Austin, TX). The electrochemical cell was composed of either 3 mm diameter glassy carbon or 2 mm diameter platinum working electrodes, a common silver-silver chloride ($\text{Ag}|\text{AgCl}$; 3.0 M KCl) reference electrode, and a coiled platinum wire counter electrode. All potentials are reported with respect to the $\text{Ag}|\text{AgCl}$ reference electrode. Platinum and glassy carbon working electrodes were successively polished using 1.0, 0.3, and $0.05 \mu\text{m}$ alumina slurries on a Metaserv 2000 grinder/polisher (Buehler; Lake Bluff, IL). Electrodes were then rinsed with purified water. Platinum electrodes were electrochemically polished in 1.0 M sulfuric acid by cycling the potential between -0.3 V and $+1.2 \text{ V}$ at 100 mV/s for 50 cycles. Electrodes were used immediately after polishing.

Evaluation of sensitivity retention.

Sensitivity retention was monitored by amperometric calibrations to track changes in H_2S response, referred hereafter as a passivation test. Passivation tests were performed via constant potential amperometry (CPA) or triple pulse amperometry (TPA). Regardless of the method, the working electrode was first polarized in 20 mL of deoxygenated 0.10 M aqueous KNO_3 (adjusted to pH 7.4) for 2000 s prior to the introduction of H_2S standards. A fresh H_2S stock solution was prepared daily by dissolving 12.0 mg $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ in 10.0 mL of deoxygenated 150 μM aqueous EDTA. The container headspace was purged with N_2 and sealed with a rubber septum. Stock solution concentration was verified by iodometric titration.²² Aliquots of the H_2S stock were injected into the stirred electrolyte solution to generate an initial calibration curve. The procedure was repeated in 20 mL of fresh KNO_3 without repolishing the electrodes for subsequent calibration curves. After a series of calibrations, sensitivities were calculated and normalized to the initial calibration sensitivity to determine performance retention under different electrochemical techniques and parameters.

Sensor performance in proteinaceous media was assessed by performing a calibration in simulated wound fluid (SWF), a solution of 10 vol% FBS in deoxygenated PBS. The test was performed following an initial calibration using the same TPA calibration procedure described above. Analytical performance was compared to a calibration performed in deoxygenated PBS.

Surface analysis of sulfur adsorption.

Plates of glassy carbon (10 mm x 30 mm x 1 mm; SPI Supplies; West Chester, PA) were used in place of disk electrodes to facilitate X-ray photoelectron spectroscopy (XPS) analysis. After the passivation test was performed on the plates, the electrode surfaces were characterized using a Kratos Axis Ultra DLD X-ray photoelectron spectrometer using a monochromatic Al K α X-ray source (base pressure = 6×10^{-9} torr). Survey scans (80 eV pass energy) and high-resolution scans (20 eV pass energy) for C 1s, O 1s, and S 2p were obtained. Atomic concentrations were calculated using Kratos Vision software.

Selectivity measurement.

Electrodes were allowed to polarize under CPA (+1.1 V) or TPA for 2000 s in 20 mL of 0.10 M KNO₃ (pH 7.4). A calibration was then determined by injecting stock H₂S solution. In fresh KNO₃, similar injections were made using stock solutions of NO (saturated, 1.9 mM), CO (saturated, 1.0 mM), sodium sulfate, sodium nitrate, L-ascorbate, acetaminophen, ammonium hydroxide, hydrogen peroxide, L-cysteine, and L-glutathione to determine sensitivities of these potential interferents. Saturated NO and CO solutions were prepared by purging $\times 20$ mL of phosphate buffered saline (10 mM, pH 7.4) with N₂ for 25 min, followed by NO or CO gas for 25 min over ice. Selectivity coefficients were calculated to compare the performance of H₂S to the potential interferent using Eq. 3:

$$\log k_{H_2S,j} = \log \left(\frac{S_j}{S_{H_2S}} \right) \quad (3)$$

where S_{H_2S} and S_j represent the sensitivities calculated for H₂S and interferent j, respectively.

Data Analysis.

Values for sensitivity retention, limit of detection, response time, and selectivity coefficients are expressed as the mean $\pm$ the standard error of the mean. Comparisons were made using a 2-tailed student t-test with $p < 0.05$ indicating statistical significance. A threshold of $R^2 > 0.99$ for the linear correlation coefficient was used to determine the linear range of the sensor.

Results and Discussion

Platinum (Pt) and glassy carbon (GC) are commonly used as working electrodes for many electrochemical sensor configurations. Both electrode materials were evaluated to determine which would be optimal for H₂S detection. Glassy carbon was found to have a more favorable (i.e., lower) oxidation potential for removing adsorbed sulfur from the electrode surface than Pt (Figure S1), potentially due to the more covalent Pt-S bond that forms upon sulfur adsorption to platinum, making sulfur stripping more difficult.²⁷ Platinum was also found to have a less reproducible sensitivity to H₂S under TPA (data not shown). For these reasons, GC electrodes were used for all subsequent work, unless otherwise mentioned.

Electrochemical characterization of hydrogen sulfide.

Previously reported CVs of H₂S collected with platinum electrodes pointed out three main features at -0.1, +0.475, and +1.0 V vs. Ag|AgCl that were attributed to the oxidation of sulfide, elemental sulfur adsorption with associated side reactions, and the oxidation of deposited sulfur to water-soluble sulfate ions (SO₄²⁻), respectively.²⁷ The proposed oxidation of adsorbed sulfur on glassy carbon is represented by Eq. 4:

At pH 7.4, roughly 20% of hydrogen sulfide remains in the undissociated form while 80% is present as HS⁻. Cyclic voltammetry (CV) of H₂S performed at pH 7.4 combines the oxidation profile of both species (Figure S1). The electrolyte pH was adjusted to 3.2 and 10.0 to individually characterize the biologically-relevant forms of hydrogen sulfide (H₂S and HS⁻, respectively). Cyclic voltammetry at both extremes demonstrates unique oxidation features for each (Figure 1). Of note, a large oxidation peak at +0.85 V with a prominent shoulder was observed at +0.85 V for pH 3.2 solutions, although the expected sulfide oxidation peak was significantly reduced relative to Pt (Figure 1). At pH 10.0, only a single oxidation peak is visible at +0.5 V. This oxidation peak was attributed to the conversion of adsorbed elemental sulfur to sulfate ions. Therefore, the oxidation of adsorbed sulfur occurs more readily for the HS⁻ versus H₂S form, as signified by the peak shift to lower potentials. As HS⁻ is the dominant species at physiological pH, the electrochemical removal of adsorbed sulfur is favorable on GC electrodes at a working electrode potential above +0.5 V (Figure S2).

Sulfur passivation during constant potential amperometry.

Adsorption of elemental sulfur deters sensor performance by reducing the available electroactive surface area of the working electrode and thus sensitivity to H₂S. Few studies have attempted to characterize the formation of the passivating layer.^{27,42} A passivation test was thus developed to monitor the formation of the sulfur passivation layer over subsequent calibration trials and provide insight into the kinetics of sulfur layer formation. Constant potential amperometry (CPA) was used at potentials both above and below the main HS⁻ CV feature at +0.5 V (0.0, +0.1, +0.3, +0.7, and +1.1 V) to evaluate how electrode passivation is affected by the applied potential. As expected, the use of potentials below the adsorbed sulfur oxidation peak (<+0.5 V) resulted in passivation due to the inability to convert the adsorbed sulfur to water soluble sulfate. At higher potentials (≥ +0.7 V), enhanced retention of the electrode's initial sensitivity was observed, though with limited test-to-test reproducibility (Figure S2).

The elemental composition of the sulfur passivation layer was next probed as a function of the working potential under CPA. Following the passivation test, carbon, oxygen, and sulfur XPS spectra were analyzed for changes that might account for the observed sensitivity variation. Regardless of the applied working potential, no changes were observed in the carbon 1s or oxygen 1s regions. However, distinct variations in the sulfur 2p region were clearly evident between +0.1 and +1.1 V (Figure 2). Two distinct features were observed in

these spectra: a doublet assignable to elemental sulfur (S^0) at 163.9 eV and a sulfate sulfur 2p peak at 168.9 eV. As the working potential was increased to +1.1 V, the ratio of S^0 to SO_4^{2-} on the electrode surface decreased, corroborating the noted increase in sensitivity retention. At higher potentials, the SO_4^{2-} readily diffuses out into the aqueous solution resulting in a more consistent electroactive surface area. Applied potentials above +0.7 V provided optimal conditions for clearing the sensor surface of elemental sulfur (Figure S3). However, potentials >+0.7 V decreased the selectivity of the sensor without consistent sensitivity retention.

Triple pulse amperometry.

The large oxidation potential (>+0.7 V) required to prevent sulfur poisoning of the electrode surface is also capable of oxidizing other interferent species found in biological environments. Constant potential amperometry at such a high potential therefore poses a substantial obstacle to accurate measurement of hydrogen sulfide. Triple pulse amperometry, a subcategory of PED, is an electrochemical technique capable of cycling between three potential pulses, with current sampling only occurring during the second half of the third pulse (Scheme 1). The initial two pulses were purposed to apply a high potential for electrode regeneration and allow the byproducts to diffuse away, respectively. The final pulse returns to a lower potential for hydrogen sulfide oxidation.

As both H_2S and HS^- are oxidized at or below +0.1 V⁴³, we opted for this working potential as the measuring pulse potential in each TPA cycle (Scheme 1, C). Electrode regeneration was performed by oxidizing adsorbed sulfur to sulfate ions at applied potentials >+0.5 V (Scheme 1, A). An intermediate pulse was used as a temporal buffer between the cleaning and measuring pulses (Scheme 1, B). By avoiding immediately oxidizing HS^- or H_2S , the intermediate pulse provided time for sulfate ions to diffuse away, assisted by coulombic repulsion forces, following the cleaning pulse. The cleaning potential and pulse lengths were systematically optimized to yield the greatest sensitivity retention and widest linear dynamic range.

A cleaning pulse potential of +0.7 V was used initially to determine the electrode regeneration efficacy of TPA relative to CPA through passivation tests. Cleaning pulse potentials at or below +0.7 V yielded no significant difference in sensitivity retention compared to CPA experiments with the working electrode held at +0.1 V. Increasing the length of the cleaning pulse per TPA cycle at these potentials resulted in negligible electrode regeneration, suggesting that the cleaning potential was insufficient (Table S1). In this regard, adsorption of sulfur generated from the low potential measuring pulse outpaced the efficacy of the cleaning pulse, resulting in rapid electrode passivation.

As the regenerative effects of CPA at +0.7 V were not observed for TPA at the same potential, the cleaning potential was increased to an overpotential of +1.1 V to further amplify the rate of adsorbed sulfur oxidation. The larger potential yielded increased sensitivity retention, sulfate production, and initial calibration sensitivity, although sensitivity loss continued to occur over additional calibrations (Figure S4). Reproducible cleaning rates were achieved through the introduction of an even larger overpotential (+1.5 V) for the cleaning pulse, but concomitantly resulted in a poor linear response range.

Optimization of the linear dynamic range was ultimately achieved by varying the ratio of time spent on the cleaning and measuring pulses per TPA cycle. Cleaning and measuring pulse lengths with ratios of 1:1 through 1:10 were tested, resulting in various linear ranges and sensitivities. A 1:2 ratio, representing a 0.5 s cleaning pulse and a 1.0 s measuring pulse, was chosen for subsequent experiments because of its high sensitivity and wide linear range with minimal increase in the total cycle time. As a result, highly linear calibration curves, increased sensitivity, and stable responses were observed over six calibrations (Figure 3).

The final optimized TPA parameters were: a +1.5 V cleaning pulse, -0.3 V intermediate pulse, and +0.1 V measuring pulse with pulse lengths of 0.5, 0.1, and 1.0 s, respectively. With these values, the calibration sensitivity (57.4 ± 13.0 nA/ μ M) was greatly increased compared to CPA at +0.1 V (3.5 nA/ μ M). While a significant decrease in CPA sensor sensitivity was observed immediately following the initial calibration trial, TPA under these parameters resulted in no significant change in sensitivity over the entirety of a passivation test (Figure 4). Although stabilization in CPA sensitivity occurred following an initial drop, the reduction in the already low CPA sensitivity lead to poor limits of detection (9.1 ± 3.0 μ M) that limit its biological utility.

Using the optimized TPA parameters, the sensor routinely encompassed a wide linear dynamic range (150 nM-15 μ M), and thus the majority of cited physiological concentrations in recent publications.^{20,37,38} Rapid response times (<10 s) were observed when aliquots of hydrogen sulfide were introduced (Table 1). However, the temporal resolution was inherently restricted to intervals of 1.6 s due to the length of a TPA cycle. Stable limits of detection in the sub-micromolar range (<100 nM) and response times were also observed to accompany the retention of sensitivity to H₂S (Table 1). Sensor response times were consistently below 10 s and comparable to commercial electrochemical, redox-mediated sensors.^{35,44} Chronoamperometry was used to determine the impact of capacitive discharge on the sampled current during the measuring pulse. Prior to current sampling, $80 \pm 6\%$ of the current was discharged. Slightly greater discharge ($88 \pm 4\%$) was observed at the end of the pulse, with negligible change to overall background current and H₂S detection.

The ratio of elemental sulfur to sulfate present on the electrode surface after consecutive TPA calibrations was also probed using XPS. The reduced intensity of the sulfate sulfur 2p peak at 168.9 eV under TPA, compared to CPA at +1.1 V in Figure 2, is attributed to the inclusion of the intermediate pulse (Figure 5). Using coulombic repulsion to drive the ions into solution, the intermediate pulse prevented the large buildup of sulfate observed for CPA H₂S detection. The increased intensity of elemental sulfur 2p peak at 163.9 eV is likely a consequence of the low potential measuring pulse producing insoluble sulfur. While initial sulfur adsorption occurs briefly, any continuous buildup is mitigated by the integrated cleaning pulse (Figure S5).

Sensor selectivity and performance in proteinaceous media.

Quantification of analytes in biological media presents numerous experimental complexities that need to be considered prior to measurement. For example, the non-specific oxidation of electroactive interferent species (e.g., NO and CO) for amperometric methods will add to the measured current response, resulting in inaccurate values and undesirable background

current contributions. Typically, selectivity is imparted to a sensor by surface modifications (i.e., permselective membranes) or through the use of working potentials below the oxidation potential of common interferents.^{45,46} To compare the selectivity of the H₂S sensor using TPA, baseline values were experimentally determined for GC electrodes using CPA. The H₂S sensors via CPA (+1.1 V) were characterized as having comparable surface regeneration capabilities to the TPA method. This potential (+1.1 V) was thus chosen as the minimum threshold for improving H₂S selectivity. The CPA-based detection at +1.1 V provided only poor to moderate selectivity against NO, CO, nitrite, acetaminophen, hydrogen peroxide, and ammonium (Table 2). As expected for a lower pulse potential (+0.1 V), significant improvement in the selectivity for H₂S over NO, CO, nitrite, hydrogen peroxide, cysteine, and glutathione was observed using TPA. Of note, sensor selectivity for acetaminophen and ammonium remained consistent (Table 2).

Given the large disparity between the CPA working potential and the measuring potential during TPA, the enhanced selectivity towards H₂S under TPA was not as great as expected. Rapidly modulating the potential during TPA may have been the culprit in the moderate improvement, as greater background current and noise resulted. In addition, potentials greater than +1.5 V have previously been shown to modify the functional groups on the surface of GC electrodes.^{47,48} Such inherent surface changes might have contributed to the disparity between TPA and CPA selectivity by altering the rate of electron transfer.

The performance of the sensors in proteinaceous media was evaluated to determine the effects of protein scavenging and biofouling. For these experiments, the sensors were simply calibrated in PBS and SWF to analyze changes in linear range, sensitivity, and LOD. Relative to PBS, sensitivity was decreased by >75% in SWF. Concomitant reductions in linear range (1-15 μ M) and LOD (>200 nM) were also observed in proteinaceous SWF media (Figure S6). The diminished sensor performance was attributed to protein fouling of the electrode, confirmed by electrode discoloration, and scavenging of the H₂S prior to diffusion to the sensor surface. While H₂S sensing via TPA proved selective over common biological interferents, the observed surface biofouling and diminished sensitivity warrant the evaluation of anti-biofouling coating compatibility to further tune sensor performance in certain biological media depending on the intended application.

Conclusions

Applying separate cleaning and measuring pulses allows for the amperometric detection of H₂S using GC electrodes without performance degradation from sulfur-induced passivation. A cleaning potential of +1.5 V enabled the removal of surface adsorbed passivating sulfur through conversion to water soluble sulfate ions, a byproduct of the electrochemical reaction. The use of TPA facilitate a sensor design with analytically useful performance parameters (i.e., stable sensitivity, rapid response time, wide linear dynamic range, and low detection limit) during six calibration trials performed over the course 7-8 hours of testing. The direct electrochemical approach eliminates the reliance on external coatings and redox mediators, freeing the sensor surface to be functionalized for more specialized applications. For example, novel and established coatings (i.e., anti-fouling permselective membranes) may present an opportunity to simultaneously increase the selectivity and reduce protein-

related biofouling of bare GC electrodes further to better accommodate H₂S sensing in more complex biological media.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Cyclic voltammograms of 0.5 mM H_2S in 0.1 M KNO_3 at pH 3.2 (solid), pH 10.0 (dash), and background (dots) on glassy carbon electrodes.

Figure 2. XPS spectra of the S 2p region from a passivation test consisting of four hydrogen sulfide calibrations conducted at +0.1 V (solid) and + 1.1 V (dash) under CPA at planar glassy carbon electrodes.

Figure 3.

Representatives response (calibration curve) of hydrogen sulfide sensors using optimized TPA parameters in 0.1 M KNO_3 (pH 7.4). Calibration sensitivity was 58.5 nA/ μM with an R^2 of 0.998. Insert: Amperometric response to the addition of hydrogen sulfide aliquots. TPA parameters are 0.5 s at +1.5 V, 0.1 s at -0.3 V, and 1.0 s at +0.1 V for the cleaning intermediate, and measuring pulses, respectively.

Figure 4. Percentage of sensitivity retained by subsequent calibrations performed using CPA at +0.1 V (triangles) and optimized TPA parameters (squares) on glassy carbon in 0.1 M KNO_3 (pH 7.4). TPA parameters are 0.5 s at +1.5 V, 0.1 s at -0.3 V, and 1.0 s at +0.1 V for the cleaning, intermediate, and measuring pulses, respectively. * $p < 0.05$

Figure 5.

XPS spectra of the S 2p region from a passivation test consisting of six calibrations conducted on glassy carbon plates. TPA parameters are 0.5 s at +1.5 V, 0.1 s at -0.3 V, and 1.0 s at +0.1 V for the cleaning, intermediate, and measuring pulses, respectively.

Scheme 1.

Potential waveform for a single TPA cycle consisting of (A) a cleaning pulse, (B) an intermediate pulse, and (C) a measuring pulse.

Table 1.

Analytical figures of merit for GC-based H₂S sensor via TPA over six consecutive calibrations during a passivation test.

Calibration Number	Retained Sensitivity (%)	Limit of Detection (nM) ^a	Response Time (s) ^b
1	100	100 ± 30	8.2 ± 2.8
2	94 ± 17	60 ± 20	6.8 ± 4.1
3	100 ± 4	40 ± 10	7.2 ± 3.3
4	102 ± 10	50 ± 30	5.1 ± 1.6
5	98 ± 7	40 ± 20	3.8 ± 1.2
6	101 ± 6	40 ± 20	6.6 ± 4.4

^aS/N=3

^bTime at 95% of the total signal

Table 2.

Selectivity coefficients for hydrogen sulfide over nitric oxide, carbon monoxide, nitrite, acetaminophen, hydrogen peroxide, ammonium, cysteine, and glutathione on glassy carbon electrodes for CPA and TPA.

Working potential (V)	Selectivity Coefficient ^a							
	Nitric oxide	Carbon monoxide	Nitrite	Acetaminophen	Hydrogen peroxide	Ammonium	Cysteine	Glutathione
+1.1 V ^b	1.49 ± 0.23	-0.86 ± 0.09	-0.61 ± 0.06	-0.82 ± 0.02	-1.35 ± 0.07	-1.54 ± 0.08	-0.92 ± 0.12	-0.92 ± 0.04
TPA ^c	-0.21 ± 0.10 [*]	-1.88 ± 0.10 [*]	-0.84 ± 0.14 [*]	-0.60 ± 0.15	-1.86 ± 0.27 [*]	-1.55 ± 0.06	-2.08 ± 0.13 [*]	-1.44 ± 0.04 [*]

^an = 3

^bConstant potential amperometry

^cCollected at optimized triple pulse amperometry parameters

^{*}*p* < 0.05 relative to +1.1 V