A HIGHLY SENSITIVE FLEXIBLE AG NPS/MWCNTS/NAFION-RU$(NH_3)_6^{3+/2+}$ ELECTRODE WITH SU-8 MICROPILLARS FOR REAL-TIME HYDROGEN SULFIDE MONITORING IN LIQUIDS

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ABSTRACT

This abstract presents a novel electrochemical sensing electrode based on a stacking layer structure of inkjet-printed Ag NPs/MWCNTs/Nafion-Ru$(NH_3)_6^{3+/2+}$ on a photo-defined flexible Kapton polyimide substrate potential for real-time hydrogen sulfide monitoring in liquid. Employing the electrochemical amperometry method, the sensing electrode shows a high sensitivity of 102.26 nA/μM/cm^2, a limit of detection (LoD) of 0.12 μM and a linear range of 0.1-1000 μM (R^2 = 0.99) with high anti-interference ability, stability and reliability in acid-base environments. Via the incorporation of highly conductive multi-walled carbon nanotubes (MWCNTs) and the SU-8 micropillars in the sensing electrode design, more than twofold sensitivity enhancement and ~85% lower LoD can be accomplished for better electrode performance. Meanwhile, this sensing electrode also shows the similar linear response in the commercial calibration kit of ELISA for the hydrogen sulfide measurement validating the effectiveness of the sensor for real-time detecting the endogenous H_2S released from living tissues, critical for monitoring cardiovascular and central nervous system-related diseases.

KEYWORDS

Flexible Sensor, Hydrogen Sulfide, Coffee Ring Effect, Sensitivity Enhancement, LoD Stacked Layer, Inkjet Printing.

INTRODUCTION

Hydrogen sulfide (H_2S) is recognized as a significant endogenous gaseous signal molecule in mammals, alongside carbon monoxide (CO) and nitric oxide (NO). This colorless and toxic gas, known for its distinct odor of rotten eggs, is produced through enzymatic catalysis involving sulfur-containing amino acids like methionine, cysteine, and cystine. Specifically, the enzymes cystathionine-β-synthase (CBS) and cystathionine γ-lyase (CSE) are involved in its synthesis. Hydrogen sulfide plays a crucial role in regulating various pathological properties and signal transmission across different tissues in mammalian systems [1]. Previous research has found that the concentration of endogenous hydrogen sulfide detected in the serum of healthy animals ranges from 30 to 100 μM, while in the brain and spinal cord, it falls between 50 and 160 μM [2]. Abnormal concentrations of hydrogen sulfide are now known to be closely associated with varieties of diseases, particularly those related to the central nervous system, e.g., Alzheimer's disease and Parkinson's disease. [3,4]. Therefore, it is essential to develop a fast, sensitive and convenient analysis method of hydrogen sulfide.

The present methods for detecting hydrogen sulfide concentrations in physiological environments often require chemical pretreatment, such as the methylene blue method [5-7] and fluorescence method [8]. Both methods operate by capturing a fixed-band light source and comparing it with the light intensity of the illuminated sample. While these methods are effective in laboratory settings with relatively clean samples, their complexity, irreversibility, and time-consuming nature make them unsuitable for real-time monitoring. In recent decades, biosensors employing electrochemical methods such as anodic stripping voltammetry (ASV) [9], ion-sensitive electrodes (ISE) [10], cyclic voltammetry (CV), and amperometry have demonstrated numerous advantages, including cost-effectiveness, high sensitivity, rapid response times, straightforward procedures, and they also hold promise for portable and real-time monitoring applications. However, practical implementation faces technical challenges. For example, during the acid or alkaline pretreatment required for ASV and ISE, acid-unstable thiol sulfides may discharge, influencing the overall concentration readings. Consequently, the results can only reflect the total sulfide content in the sample. On the other hand, amperometric sensors measure the redox current by applying a fixed potential for a period of time in the tested sample and estimate hydrogen sulfide concentration without pretreatment, enabling direct measurement [11-13]. Thus, in this paper, we will present a solid-state sensor structure based on the amperometry method for the real-time H_2S detection.

Previously, we developed a size-scalable two-electrode amperometric sensor using inkjet-printed Ag conductive electrodes. The working electrode (WE) consists of stacked rGO/Nafion-Ru$(NH_3)_6^{3+/2+}$ layers, while a chlorination process selectively forms a Ag/AgCl(s) reference electrode (RE) on the other electrode. The sensor can exhibit a linear sensitivity of 40 nA/μM/cm^2 and a LoD of 0.75 μM capable of detecting the endogenous H_2S released from living tissues and has been successfully applied for the continuous detection of H_2S released from cysteine in heating eggs. "Previous studies have highlighted the unique physicochemical and photoelectric properties of carbon nanotubes (CNTs), which enable more sensitive biosensor detection [14]. Specifically, CNT-based electrochemical transducers significantly enhance the performance of amperometric enzyme electrodes due to their highly efficient electron transfer between the underlying electrode and the redox centers of enzymes [15]. To further enhance our previous sensor performance, we propose a stacked layer structure, i.e., inkjet-printed Ag NPs/MWCNTs/ Nafion-Ru$(NH_3)_6^{3+/2+}$ on a photo-defined flexible Kapton polyimide substrate, This design leverages the high electrical conductivity of MWCNTs and increases the sensing area through the coffee-ring effect [16] on the printed sensing materials covering the sidewalls of SU-8 micropillars. We anticipate that this configuration will offer higher sensitivity and a lower limit of detection (LoD), making it suitable for monitoring diseases related to the cardiovascular and central nervous systems.

SENSOR DESIGN AND FABRICATION

The sensor fabrication basically follows our previous developed flexible inkjet printing printing process steps [17]. A 20nm copper layer was sputtered first on a flexible Kapton polyimide substrate, followed by photo-patterning photoresist as the mold for electroplating gold as the electrical feedthrough and three electrode pads. For the reference electrode fabrication, a silver layer electroplated on one of the glod pads was immersed in a 0.1 M $FeCl_3$ solution for 5 min and then in a 3 M KCl for 24 hr to form a stable $Ag/AgCl_{(s)}$ reference electrode. After the Chronication process, a 30

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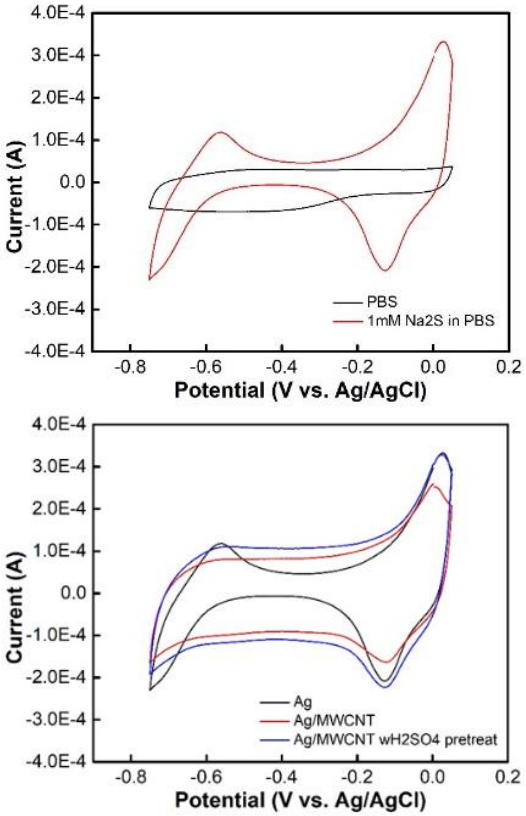

Figure 1: CV measurements of of the printed Ag (upper) and the printed Ag/MWCNTs with/without H_2SO_4 pretreatment electrodes (lower) vs. the commercial RE.

μm thick SU8-is then photo-patterned to define the electrode pads and to protect the rest of the interconnect region, which is followed by the deposition of a PU-based ionic selective membrane on the reference electrode using Dimatix DMP-2831 inkjet printer, Fujifilm Dimatix Inc. Once the reference electrode is fabricated, subsequent inkjet printing was performed to form the stacked Ag NPs/MWCNTs/Nafion-Ru$(NH_3)_6^{3+/2+}$ layer using the inks including the commercial Ag nanoparticle ink, 1 wt % multi-wall carbon nanotubes solution and the 0.4 g/L mixture of $Ru(NH_3)_6Cl_3$ nanoparticles, 0.5% Nafion, and a solution with a ratio 1:1 of D.I. water versus 80% ethanol on the working electrode. At final, the three-electrode pads weres connected to Autolab PGSTAT204 electrochemical workstation for electrical measurement.

RESULTS AND DISCUSSION

Due to the fact of hydrophobic characteristic of CNTs, the nanotubes often suffer from aggregating, winding and curling problems. Mohajeri, et al [18] proposed an electrochemical method by pretreating CNT's surface in a 0.2 M sulfuric acid with cyclic voltammetry scanning. As a result, the tubes can be cut into smaller fragments with defects in the tube end to form a carboxyl structure (-COOH), thereby enhancing the solubility and uniformity of the printed MWCNTs for accelerating electron transfer efficiency on the electrode surface. Figure 1 depicts the cyclic voltammetry (CV) measurement of both printed Ag and Ag/MWCNTs electrodes with and without H_2SO_4 pretreatment in pH 7.4 phosphate-buffered saline (PBS) solutions supplemented with or without Na_2S. The upper figure shows the Ag electrode exhibits no oxidation current in PBS solution and an oxidation current peak once the PBS is added

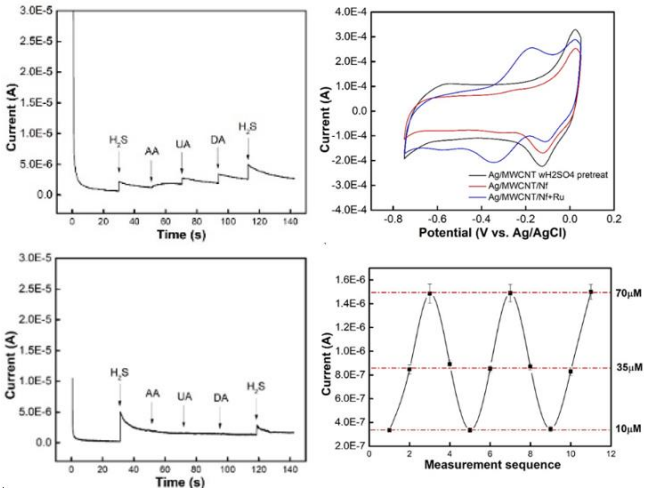

Figure 2: The titration test of the printed Ag NPs/pretreated MWCNTs with/without the Nafion cover electrodes: the saturated oxidation current measured in a pH = 7.4 PBS with the sequential addition of AA, UA, DA, and Na_2S (left) and CV (upper right) and repeated tests (lower right) of the stacked Ag NPs/MWCNTs/Nafion-Ru$(NH_3)_6^{3+/2+}$ electrode.

with Na_2S resulting from the formation of silver sulfide. In addition, Higher oxidation current peaks shown in the lower figure validate that both the Ag and Ag/pretreated MWCNTs electrodes can exhibit the same redox reaction with H_2S at 0V.

Nafion is a perfluorinated ionomer with a negative charge, which effectively repels anionic interferents from its surface. Previously, we successfully developed a Nafion-nanoparticle nanocomposite layer within the sensing electrode design, enhancing sensor sensitivity while maintaining anti-interference capabilities. Figure 2 depicts the titration test results of the printed Ag NPs/pretreated MWCNTs electrodes with and without the Nafion cover. The test involved measuring the saturated oxidation current in a pH = 7.4 PBS solution with sequential additions of 100 μM ascorbic acid (AA), 100 μM uric acid (UA), 50 μM dopamine (DA), and 20 μM Na_2S (left panel), cyclic voltammetry (CV) analysis (upper right panel), and repeating tests (lower right panel) of the

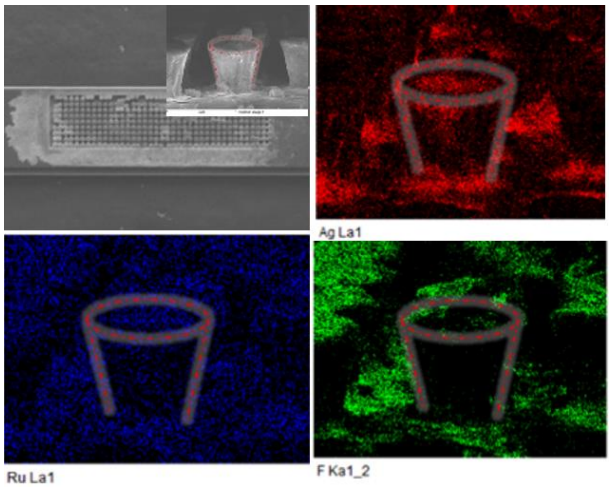

Figure 3: SEM and EDX images of the electrode with the micropillars where red, blue, and green dots indicates the signals of Ru, Ag and F atoms from Ag NPs and Nafion-Ru$(NH_3)_6^{3+/2+}$.

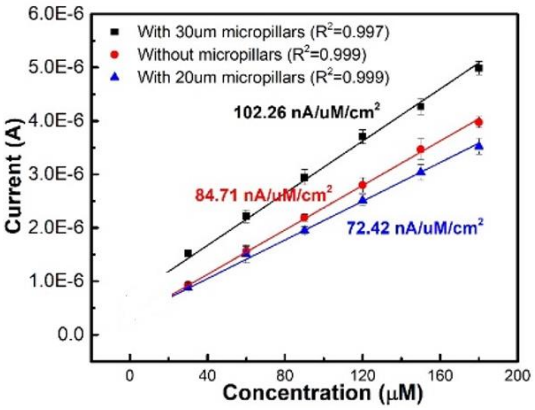

Figure 4: Sensitivity measurement of the stacked Ag NPs/MWCNTs/Nafion-Ru(NH$_3$)$_6^{3+/2+}$ electrode with/without the SU-8 micropillar design.

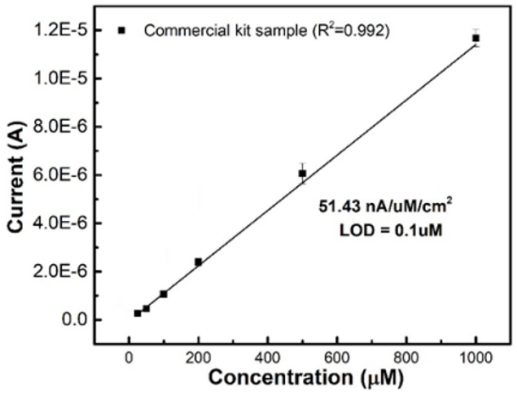

Figure 5: Sensitivity measurement of the stacked Ag NPs/MWCNTs/Nafion-Ru(NH$_3$)$_6^{3+/2+}$ electrode using the ELISA standard kit for H_2S detection.

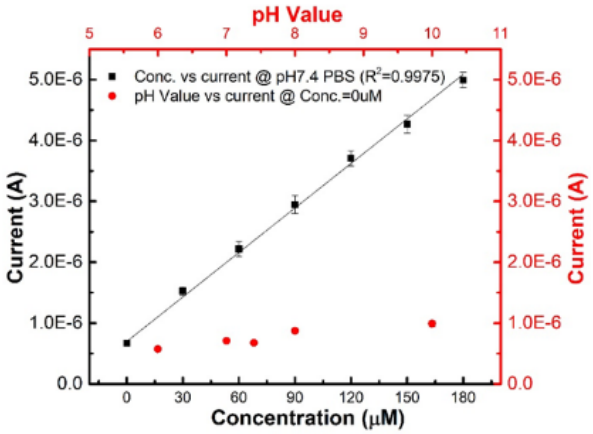

Figure 6: The current response of the Ag NPs/MWCNTs/Nafion-Ru(NH$_3$)$_6^{3+/2+}$ electrode in different PBS solutions with different pH values @ 0V bias .

stacked Ag NPs/MWCNTs/Nafion-Ru(NH$_3$)$_6^{3+/2+}$ electrode. The current and CV responses of the Ag NPs/pretreated MWCNTs with and without the Nafion cover are presented, demonstrating the effective anti-interference characteristics and oxidation current compensation achieved by incorporating the Nafion-Ru(NH$_3$)$_6^{3+/2+}$ composite, as shown in the upper right figure. Furthermore, repeating measurements exhibit consistent responses, highlighting the excellent reliability of the stacked Ag NPs/MWCNTs/ Nafion-Ru(NH$_3$)$_6^{3+/2+}$ electrode in PBS solutions with varying sulfide concentrations ranging from 10 to 70 μM/L.

The coffee-ring effect was initially proposed by a research team from the University of Chicago in 1997 [16]. This phenomenon occurs due to an imbalance in solvent evaporation rates. Specifically, the solvent near the edges evaporates more quickly than in the center, leading to an inward flow of liquid to replenish it. This inward flow carries solutes, resulting in the accumulation of solutes at the edges and a gradual decrease in thickness from the center towards the edges. Thus, we took the aforementioned process step of photo-patterning SU-8 to form SU-8 micropillars on the sensing electrode. Fig. 3 presents SEM and energy-dispersive X-ray spectroscopy (EDX) images of the electrode with micropillars, confirming the expected distribution of sensing materials all over the pillars, especially in the sidewall of the pillars resulting from the coffee-ring effect as expected. Fig. 4 illustrates the sensitivity measurement of the Ag NPs/MWCNTs/Nafion-Ru(NH$_3$)$_6^{3+/2+}$ electrode with and without the SU-8 micropillar design. The electrode demonstrates a sensitivity of 84.71 nA/μM/cm^2 and a LoD of 0.08 μM, which can be further enhanced to 102.26 nA/μM/cm^2 and LoD of 0.12 μM, respectively while incorporating a 30 μm tall micropillar design.

The electrode's performance is further assessed using the enzyme-linked immunosorbent assay (ELISA) standard kit for H_2S detection, as shown in Fig. 5. A highly linear output current response, i.e., R^2 = 0.99 to H_2S concentration indicates the sensor can well perform the detection of H_2S. The observed lower sensitivity, i.e., 51.43 nA/μM/cm^2 characterized using the kit could be attributed to sample preparation issues, as H_2S might undergo oxidation by dissolved O2 to form sulfur. Thus, how to correlate the current response with the real concentration of H_2S for accurate sensing in the solution is still underway.

In the sample preparation of the PBS added with sodium sulfide, the pH value of the solution will change with the concentration of the sulfide as the following equation:

$$Na_2S_{(aq)} + H_2O_{(l)} \rightarrow NaHS_{(aq)} + NaOH_{(aq)} \quad (1)$$

The solution tends to become more alkaline, with the pH value rising from 7.42 to 9.54 as the sodium sulfide concentration in the PBS increases from 0 to 1400 μM. The pH value, however, changes by less than 2% in the PBS containing hydrogen sulfide within the physiological range. To assess the sensor's response, we tested the printed sensing electrode in buffer solutions with pH values of 6.0, 7.0, 7.4, 8.0, and 10.0, respectively. The electrode consistently maintained current output, as indicated by the red dots in Fig. 6, demonstrating good sensor specificity unaffected by pH changes in the testing environment. As compared to the other sensing electrodes listed in Table 1 [2-5], the proposed stacked Ag NPs/MWCNTs/Nafion-Ru(NH$_3$)$_6^{3+/2+}$ electrode exhibits high sensitivity, a wide detection range, and a low limit of detection (LoD) without requiring any sample retreatment, showcasing its potential for clinical research applications.

CONCLUSION

We have successfully demonstrated a flexible electrochemical hydrogen sulfide sensing electrode based on a stacking layer structure of inkjet-printed Ag NPs/MWCNTs/ Nafion-Ru(NH$_3$)$_6^{3+/2+}$ on a e Kapton polyimide substrate. Experimental results show the electrode incorporated with sulfuric acid-pretreated MWCNTs and SU-8 micropillars can exhibit a higher sensitivity, a lower LoD and a wider linear range from 0.1 to 1000 μM with high anti-interference ability, stability and reliability in acid-base environments. Besides, the sensing electrode shows the similar

Table 1: The characteristic comparison of the developed metamaterials.

Reference	Working Electrode	Electrolyte	Applied voltage	Sensitivity ($nA\mu M^{-1}cm^{-2}$)	LOD (μM)	Linear range ($\mu M\ L^{-1}$)
Panda, et al. [11]	PtNi NPs	0.05M PBS (pH=7.0)	+0.49V	323	0.004	0.0125–1031
Wang, et al. [12]	ITO	$Ru(NH_3)_6^{3+}$ with 0.1M PBS (pH=7.4)	+0V	3.5	0.17	0.5-10
Jeromiyas, et al. [13]	GCE/RGO-MoS2/ P-OPD	0.1 M PBS (pH 7.4)	+0.15V	11	0.01	0.1-790
Aziz, et al. [19]	ITONP/APTMS/ITO	Equal volumes of 0.1 M KNO_3 and 50 mM Tris buffer (pH=8)	+0.3V	187.3	3	10-1000
Tsou et al. [16]	Ag NPs /RGO/ Nafion+ $Ru(NH_3)_6Cl_3$	0.1 M PBS (pH=7.4)	+0V	59.71	0.26	N/A
This Work	**Ag NPs /MWCNTs/ Nafion+ $Ru(NH_3)_6Cl_3$**	**0.1 M PBS (pH=7.4)**	**+0V**	**102.26**	**0.12**	**0.1-180**
		Elisa standard kit		**51.43**	**0.1**	**0.1-1000**

linear response in the commercial calibration kit of ELISA for the hydrogen sulfide measurement indicating the sensing electrode with appropriately correlate the current response with the real concentration of H_2S has the potential for real-time detecting the endogenous H_2S for clinical cardiovascular and central nervous system-related disease diagnosis.

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