EAM
Environmental and Materials
EAM 3(1): 1–14
ISSN 3025-0277

Institute for Advanced Science, Social and Sustainable Future
MORALITY BEFORE KNOWLEDGE

Preliminary study of screen–printed gold electrode for H_2O_2 sensor based on electrochemiluminescence of luminol

Junjunan Muhammad Syukur[1,*], Afiten Rahmin Sanjaya[1], Isnaini Rahmawati[1], Muhammad Ridwan[1]

[1] *Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, West Java 16424, Indonesia.*

*Correspondence: junjunan.muhammad@sci.ui.ac.id

Received Date: February 1, 2025 Revised Date: February 22, 2025 Accepted Date: March 25, 2025

ABSTRACT

Background: Hydrogen peroxide (H_2O_2) is mostly used in the water and dairy industries for sterilization and preservation purposes. However, excessive H_2O_2 residues in milk and tap water pose a health risk. Therefore, accurate measurement of H_2O_2 residue is essential. **Methods**: This study explores the potential of a screen–printed gold electrode (SPGE) as a sensor for H_2O_2 sensor using the electrogenerated chemiluminescence (ECL) method of luminol in the electrolyte of phosphate buffer solution (PBS) under alkaline condition (pH of 9). **Findings:** The detection of H_2O_2 was achieved a linear calibration equation of $y = 0.0215[H_2O_2] + 0.2006$ within a concentration range of 0.5 to 200 μM ($R^2 = 0.9998$), demonstrating reliable ECL measurements. **Conclusion**: The analytical performance evaluation of H_2O_2 sensor exhibited a low limit of detection (LOD) of 3.06 μM, a limit of quantification (LOQ) of 10.20 μM, and good measurement repeatability, with a relative standard deviation (%RSD) of 6.03%, which is below ⅔ of the Horwitz coefficient of variation (9.85%). Unmodified SPGE offers simplicity, ease of use, a stable surface, and good conductivity while maintaining excellent performance. **Novelty/Originality of this article:** The application of the ECL method on SPGE for H_2O_2 detection offers excellent analytical performance, making it a promising approach for monitoring H_2O_2 residues in the water and dairy industries, with a recovery from 83.83 to 106.01%.

KEYWORDS: electrochemiluminescence; gold; hydrogen peroxide.

1. Introduction

H_2O_2 is a strong oxidizing and reducing chemical compound widely used in water treatment plants through advanced oxidation processes to decompose organic and inorganic contaminants (Rayaroth et al., 2022; Rismetov et al., 2014). In the dairy industry, H_2O_2 is employed as a disinfectant and preservative by activating the lactoperoxidase system (Ivanova et al., 2019). However, H_2O_2 residual can cause damage to macromolecules, necessitating strict regulation of its permissible levels (Syukur et al., 2023).

Several methods for H_2O_2 detection have been successfully and reliably reported, including high-performance liquid chromatography (HPLC) (Ivanova et al., 2019) and chemiluminescence sequential injection analysis (SIA) (Jones & Lee, 2019). However, these methods often require long measurement times, involve high costs, and demand extensive maintenance (Syukur et al., 2023). The limitations can be addressed by the electrogenerated chemiluminescence (ECL) method that combines electrochemical and optical methods,

Cite This Article:
Syukur, J. M., Sanjaya, A. R., Rahmawati, I., & Ridwan, M. (2025). Preliminary study of screen–printed gold electrode for H2O2 sensor based on electrochemiluminescence of luminol. *Environmental and Materials, 3*(1), 1-14. https://doi.org/10.61511/eam.v3i1.2025.1656

making it a powerful device for rapid and precise measurements. Its advantages, including high sensitivity, low background noise, and simple instrumentation, make ECL a promising alternative for H_2O_2 detection (Rahmawati et al., 2021).

The ECL is a unique technique that combines an optical sensor (chemiluminescence) with an electron transfer process occurring on the electrode surface (Rahmawati, Einaga, et al., 2022). Luminophore agents such as luminol (Irkham et al., 2021; Rahmawati, Saepudin, et al., 2022), $Ru(bpy)_3^{2+}$ (Fiorani et al., 2019; Irkham et al., 2016, 2020), and quantum dots (Valenti et al., 2018) are used to generate photon emission through redox reactions. Luminol is selected due to its widespread application as a chemiluminescent agent, offering advantages such as low cost, chemical safety, and better solubility in alkaline aqueous conditions (Rahmawati et al., 2021). The ECL of luminol is produced through two main pathways: annihilation and co-reactant pathways (Rahmawati et al., 2024). The annihilation pathway produces strong light emission, this pathway involves aprotic and deoxygenated solvents for example, DMSO and *p*-benzoquinone (Garcia-Segura et al., 2012). Meanwhile, the co-reactant pathway involves chemical species such as H_2O_2 (Fatah et al., 2024), $S_2O_8^{2-}$ (Irkham et al., 2016), $C_2O_6^{2-}$ (Irkham et al., 2021), TPrA and $C_2O_4^{2-}$ (Valenti et al., 2018), which produce radicals that react with luminophore species in electrochemical reaction, resulting in strong ECL intensity (Irkham et al., 2024). This pathway is compatible with aqueous solvent, enabling multi-analyte detection of various substances, including H_2O_2 (Rahmawati et al., 2025), NaClO (Rahmawati et al., 2024), Glucose (Tian et al., 2017), lactate (Bhaiyya et al., 2021), cholesterol (Carvajal et al., 2012), pesticide (Irkham et al., 2024), heavy metal (Harmesa et al., 2024), immunoassays (Rahmawati et al., 2021) and biosensors (Fiorani et al., 2019; Rahmawati, Einaga, et al., 2022).

Gold electrodes have been widely used for detecting glucose, cholesterol, and H_2O_2 by the ECL method (Ballesta-Claver et al., 2011; Liu et al., 2008; Syukur et al., 2023). Gold is an inert material with excellent conductivity, high stability (Koç et al., 2021), low background current (Syukur et al., 2023), and enhanced activity in base condition at oxidation potential (Prehn et al., 2012). Additionally, gold electrodes can be modified with different structures (Dehdari Vais & Heli, 2016), thiol (Rong et al., 2010), and poly-luminol coating for various analytical applications (Carvajal et al., 2012). Gold possesses distinct characteristics in the form of nanowires (Patella et al., 2024), nanolayers (Dehdari Vais & Heli, 2016), and modifications with polyamic acid (Zamfir et al., 2016) or 1,4-benzoquinone (Laroussi et al., 2021), which can be immobilized using electrochemical methods to achieve a low limit of detection (LOD) for H_2O_2 detection. However, these modifications are costly and complicated to implement. Screen-printed electrodes (SPEs) for point-of-care testing have unique properties, including miniaturization, cost-effectiveness, high sensitivity, and fast measurement capability (Syukur et al., 2023). In this preliminary study, the combination of SPEs with gold (SPGE) is explored as a promising approach for H_2O_2 detection using the ECL method. This approach pulls the advantages of both SPE technology and gold's superior electrochemical properties, providing insights into its potential application for future analytical developments.

2. Methods

2.1 Materials

Analytical grade materials used were luminol (3-aminophthalhydrazide) supplied from Tokyo Chemical Industry, while potassium ferricyanide ($K_3[Fe(CN)_6]$) was supplied from WAKO. 30% hydrogen peroxide (H_2O_2), and all other chemicals were supplied from Sigma-Aldrich. A 10 mM stock solution of luminol was prepared by dissolving luminol in 0.1N NaOH. Double-distilled water was obtained using Direct-Q3 UV system manufactured by Merck Millipore.

2.2 Instrumentations

The electrochemical measurements were carried out using potentiostat(PGSTAT204) with connector (DSC) and SPGE (250AT) from Metrohm with a gold, a silver and a platinum were used as working, reference, and auxiliary electrodes, respectively. The ECL signals were detected using a photomultiplier tube (PMT) (H11902-20) from Hamamatsu with instrumentation system from a published journal (Syukur et al., 2023).

2.3 Electrochemically active surface area (ECSA) study

The ECSA of SPGE were studied by dropping 40μL of 1mM $K_3[Fe(CN)_6]$ in the electrolyte of 0.1M phosphate buffer solution (PBS) (pH 9) using cyclic voltammetry (CV) technique from –0.4 to +0.6 with various scan rates from 0.050 to 0.175 V/s. The ECSA was obtained using the Randles-Sevcik formula in equation 1 (Annisa et al., 2024):

$$A = \frac{i_p}{2.69x10^5.n^{\frac{3}{2}}.D^{\frac{1}{2}}.C.V^{\frac{1}{2}}} = \frac{K}{2.69x10^5.n^{\frac{3}{2}}.D^{\frac{1}{2}}.C} \qquad \text{(Eq. 1)}$$

Where, A is the surface area of electrode, n is the electrons transferred number, ip is the peak current, D is the diffusion coefficient of $K_3[Fe(CN)_6]$ ($D = 7.6 \times 10^6$ cm^2 s^{-1}), C is the concentration, V is the scan rate and K is slope of plot ip vs $V^{½}$.

2.4 ECL of luminol – H_2O_2 study

The ECL behavior of SPGE was carried out by dropping 40μL of 1 mM luminol with various concentrations of H_2O_2 solution in 0.1M PBS (pH9) using CV technique with scan rates from 0.1 V/s. During CV measurement, the ECL intensity was detected by using the PMT voltage of 0.8 V. The ECL instrument setup for H_2O_2 detection is shown in Figure 1.

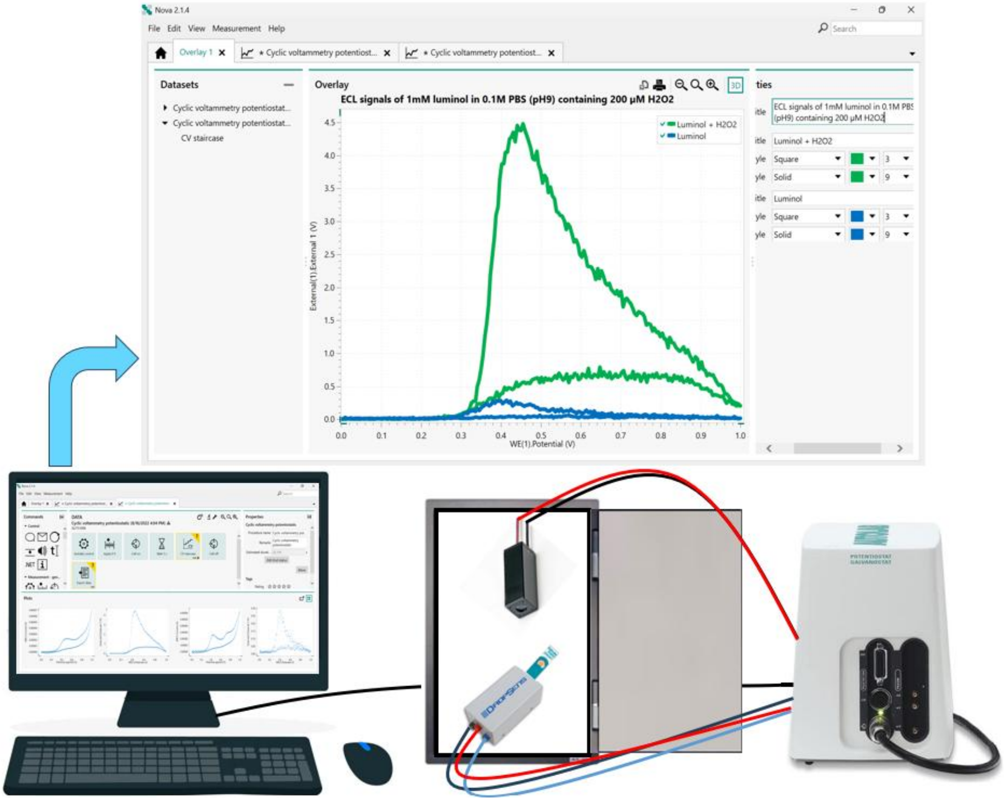

Fig. 1 The ECL instrument setup for H_2O_2 detection

3. Results and Discussion

3.1 The electrochemical behavior of SPGE

The electrochemical behavior of SPGE surface was studied using 1mM $K_3[Fe(CN)_6]$ in 0.1 M PBS as the electrolyte at various scan rates to explore the redox activity of the electroactive species. The reaction was found to be reversible, as shown in Figure 2a. The oxidation-reduction reaction of $K_3[Fe(CN)_6]$ is as follow:

$$[Fe(CN)_6]^{3-} + e \rightleftharpoons [Fe(CN)_6]^{4-} \qquad \text{(Eq.2)}$$

The oxidation current value of $[Fe(CN)_6]^{3-}$ (Ipa) was observed at approximately +0.2 V, while the reduction current value of $[Fe(CN)_6]^{4-}$ (Ipc) appeared around +0.05 V. These values were obtained to explore whether the electron transfer mechanism was diffusion-controlled or adsorption-controlled. The oxidation and reduction current of SPGE were higher than those of the screen-printed carbon electrode (SPCE), indicating that SPGE exhibited greater conductivity (Figure 2b).

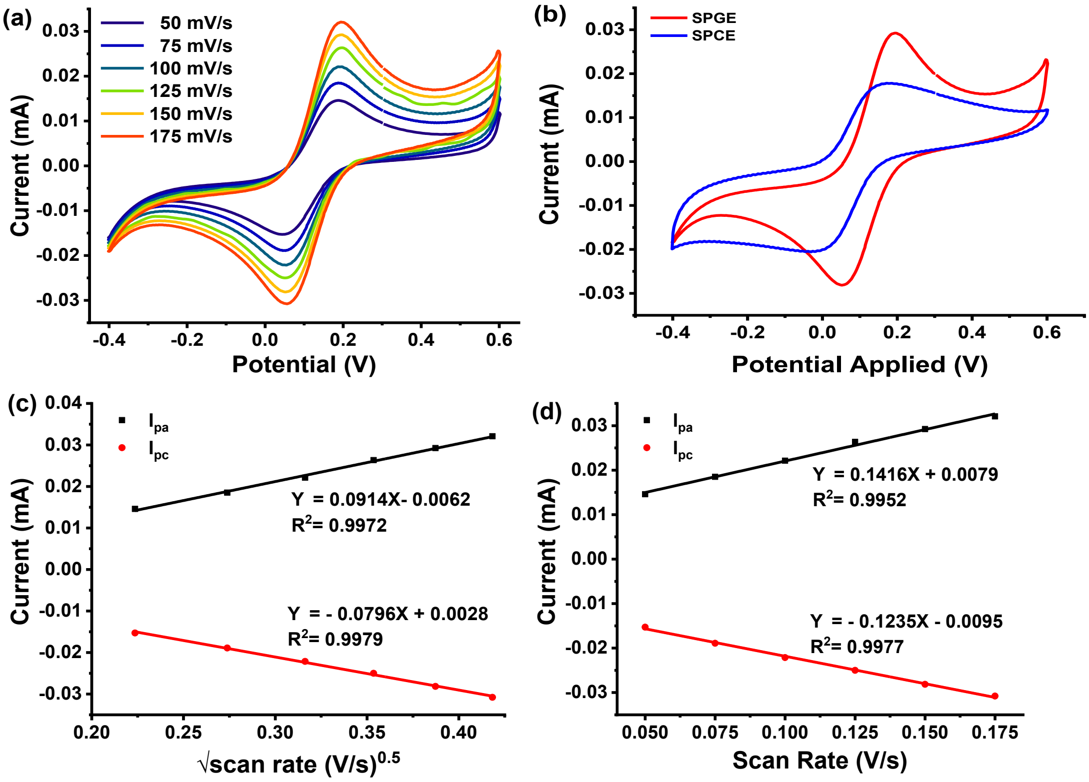

Fig. 2 (a) Cyclic Voltammogram of 1mM $K_3[Fe(CN)_6]$ in 0.1M PBS (pH9) at various scan rates on the SPGE surface; (b) Comparison of CV responses of 1mM $K_3[Fe(CN)_6]$ on SPGE and SPCE; (c) Calibration curve using Current vs. √scan rate plot; (d) Current vs. scan rate plot on SPGE

The dependence of √scan rate ($(V/s)^{0.5}$) plot (x-axis) and Ip (y-axis), shown in Figure 2c, defines the electron transfer mechanism as diffusion-controlled. This was confirmed by the linearity of the Ipa - $(V/s)^{0.5}$ plot ($R^2 = 0.9972$), which was higher than that of the Ipa - V/s plot ($R^2 = 0.9952$) (Figure 2d). The ECSA of SPGE was determined using the slope of the Ipa - $(V/s)^{0.5}$ plot, calculated with the Randles-Sevcik equation. The ECSA of SPGE was found to be 12.33 mm^2, which was significantly larger than the ECSA of SPCE (6.07 mm^2) (Syukur et al., 2023).

3.2 The Mechanism of luminol ECL on the SPGE surface for H_2O_2 detection

The ECL and CV signals of luminol with and without H_2O_2 in the PBS electrolyte on SPGE are shown in Figure 3. The CV signals of H_2O_2 and PBS indicate the oxidation of the gold electrode, forming gold (III) hydroxide ($Au(OH_3)$ in a basic condition, at approximately +0.6 V as an insert in Figure 3a. Additionally, the oxygen evolution reaction (OER) occurs beyond +0.8 V by producing oxygen. The reverse potential at +0.15 shows the reduction of $Au(OH)_3$ (Cui et al., 2004; Lović et al., 2017; Prehn et al., 2012; Syukur et al., 2023). The ECL signals of H_2O_2 and PBS exhibit noise signals, therefore, H_2O_2 cannot be measured without luminol. In a basic condition (pH 9), luminol dissociates in the presence of hydroxide, forming luminol anions. The CV signals show the oxidation of luminol anions (L^-) with or without H_2O_2, producing luminol oxyl radicals ($L^{\bullet -}$) at an initial potential of 0.28 V, reaching a maximum potential of around 0.4 V (Cui et al., 2004). The oxidation peak of luminol anions in the presence of H_2O_2 exhibits a decrease in current peak due to the competition between luminol anion oxidation and H_2O_2 at 0.49 V, which adsorbs onto the gold surface electrode. Additionally, the formation of $Au(OH)_3$ adsorption is observed from +0.6 to +0.8 V (Díaz-Ortega et al., 2014; Gerlache et al., 1997). The reduction current peak of $Au(OH)_3$ decreases, as $Au(OH)_3$ reacts to generate $L^{\bullet -}$ when the potential is reversed (Wahyuni et al., 2015). The electrocatalytic mechanism involving the electrolyte, luminol, and H_2O_2 can be described as follows: (Cui et al., 2004; Garcia-Segura et al., 2012; Gerlache et al., 1997):

$LH_2 + OH^- \rightarrow LH^- + H_2O$
$LH^- \rightarrow L^{\bullet -} + H^+ + e^-$ $E_p = +0.40$ V
$H_2O_2 \rightleftharpoons HO_2^- + H^+$
$HO_2^- \rightarrow HO_2^{\bullet} + e^- \rightleftharpoons O_2^{\bullet -} + H^+$ $E_p = +0.49$ V
$4\,OH^- \rightarrow O_2 + 2H_2O + 4e^-$ $E_p = +1.0$ V
$Au + 3OH^- \rightarrow Au(OH)_3 + 3e^-$ $E_p = +0.6$ V
$2Au(OH)_3 + 2e^- \rightarrow 2Au + 2H_2O + O_2 + 2OH^-$ $E_p = +0.15$ V

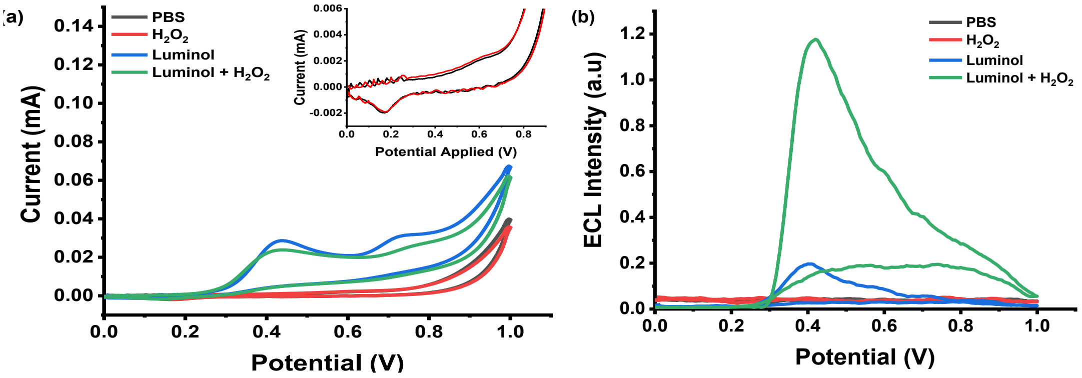

Fig. 3 (a) Cyclic Voltammogram; (b) ECL signals of 1mM luminol in 0.1M PBS (pH9) containing 50 µM H_2O_2 on the surface SPGE

The ECL of luminol with or without H_2O_2 occurs via two distinct pathways. The ECL of luminol without H_2O_2 begins with the formation $L^{\bullet -}$ at an initial potential 0.28 V (ECL^1). These radicals react with the dissolved oxygen (O_2), generating superoxide radical anion ($O_2^{\bullet -}$) and diazo quinone (L) through a propagation reaction. The ECL^1 signal increases significantly due to nucleophilic attack between $L^{\bullet -}$ and $O_2^{\bullet -}$ at carbonyl group, forming luminol endoperoxide (LOO^{2-}). A further nucleophilic attack by peroxide on another carbonyl group leads to the formation of $LOO^{\bullet -}$ followed by the loss of an N_2 bond. The resulting LOO^{2-} species undergoes decomposition to form 3-aminophthalate dianion (AP^{2-}) in its excited triple state ($AP^{2-*}(T_1)$), which subsequently transitions into the excited single state ($AP^{2-*}(S_1)$) via spin-flip process by a high-energy electron transfer reaction, as the excited electron returns to its ground state ($AP^{2-*}(S_0)$), ECL emission is produced at a wavelength of 425 nm (Garcia-Segura et al., 2012). During the reverse potential sweep, the second ECL pathway (ECL^2) occurs due to the $Au(OH)_3$ adsorption on the electrode surface. The $Au(OH)_3$ species interacts with $L^{\bullet-}$ radicals via nucleophilic attack, while OER simultaneously produces $O_2^{\bullet-}$ through a propagation reaction with $L^{\bullet-}$. this process regenerates metallic gold (Au) and produces ECL intensity via the transitions of $AP^{2-*}(S_0)$ (Cui et al., 2004).

The ECL intensity of luminol in the presence of H_2O_2 was significantly higher than in its absence (Rahmawati, Saepudin, et al., 2022), This enhancement was primarily attributed to the ECL^1 reaction, where $L^{\bullet-}$ reacts with $O_2^{\bullet-}$, which originates from the oxidation of HO_2^-. The propagation reaction of O_2 and the reaction of L with HO_2– to produce luminol hydroperoxide ($LOOH^-$) before the oxidation potential of HO_2^- is reached. The effect of $Au(OH)_3$ adsorption on ECL^2 was minimal, as its intensity merged with the ECL contribution from OER effect. Therefore, the ECL^1 and ECL^2 were selected for H_2O_2 measurement. The ECL mechanisms of luminol in the absence and presence of H_2O_2 can be described as follows (Cui et al., 2004; Garcia-Segura et al., 2012):

ECL^1:
$L^{\bullet-} + O_2 \rightarrow L + O_2^{\bullet-}$
$L^{\bullet-} + O_2^{\bullet-} \rightarrow LOO^{\bullet-} \rightarrow LOO^{2-} \rightarrow AP^{2-*}(T_1) + N_2$
$L + HO_2^- \rightarrow LOOH^- \rightarrow AP^{2-*}(T_1) + N_2$
ECL^2:
$Au(OH)_3 + L^{\bullet-} + OH^- \rightarrow Au + N_2 + AP^{2-*}(T_1) + 2H_2O$
$L^{\bullet-} + O_2 \rightarrow L + O_2^{\bullet-}$ (O_2 from OER)
$L^{\bullet-} + O_2^{\bullet-} \rightarrow LOO^{\bullet-} \rightarrow LOO^{2-} \rightarrow AP^{2-*}(T_1) + N_2$
ECL excited reaction:
$AP^{2-*}(T_1) \rightarrow AP^{2-*}(S_1) \rightarrow AP^{2-*}(S_0) + hv$

3.3 The analytical performance of H_2O_2 detection

The analytical performance of the H_2O_2 sensor's linearity was evaluated by measuring the ECL signal of 1 mM luminol in solutions with varying H_2O_2 concentrations (0, 0.5, 1, 5,10, 25, 50, 100, 200) in 0.1M PBS (pH9) as shown in Figure 4a and 4b as an insert. The calibration curve of ECL^1 (Figure 4c) displayed the linear equation of ECL^1 intensity was obtained $y = 0.0215[H_2O_2] + 0.2006$, while the ECL^2 intensity (Figure 4d) of $y = 0.0033[H_2O_2] + 0.0423$. The sensitivity of ECL^1 was better than ECL^2 due to the slope value of ECL^1 was higher. The sensitivity of ECL^1 of 17.14 a.u μM^{-1} dm^{-2}, while ECL^2 of 2.63 a.u μM^1 dm^{-2}. The LOD was achieved at ECL^1 of 3.06 µM and ECL^2 of 5.43 µM, while the LOQ was achieved at ECL^1 of 10.20 µM and ECL^2 of 18.11 µM. The result of sensitivity, LOD, and LOQ indicated that ECL^1 was selected to measure H_2O_2 due to better performance and the highest ECL signals. ECL^1 is the primary reaction between luminol and H_2O_2, while ECL^2 occurs due to the effect of $Au(OH)_3$ adsorption, OER, and residual $L^{\bullet-}$. The comparison of H_2O_2 detection at various methods and electrodes can be seen in Table 1.

Table 1. The comparison of H_2O_2 detection at various of methods and electrodes

Electrodes	method	LOD (µM)	Conc. range (µM)	ref
Pedot/rGO/AuNPs/HRP/SPGE	CA	0.08	0.5 – 200	(Mercante et al., 2017)
Au (Poly)	CA	10.00	10 – 60000	(Miah & Ohsaka, 2006)
nAu/Au	CA	7.90	50 – 800	(Dehdari Vais & Heli, 2016)
Benzoquinone/Au	CV CA	4 2.3	100 – 3000 100 – 500	(Laroussi et al., 2021)
Au Nano Wire	CA	3.21	10 – 10000	(Patella et al., 2024)
PB/BDD-SPE	CA	4.92	40 – 100	(Rahmawati et al., 2025)

BDD-SPE	ECL	2.59	1 – 100	(Rahmawati et al., 2024)
Au-PANI/BDD-SPE	ECL	2.08		
Luminol-AuNP/cys/Au	ECL	0.10	0.3 – 1000	(Cui et al., 2007)
SPCE	ECL	7.53	0.5 – 200	(Syukur et al., 2023)
Au /SPCE	ECL	4.78		
SPGE (ECL^1)	ECL	3.06	0.5 – 200	This Work
SPGE (ECL^2)		5.43		

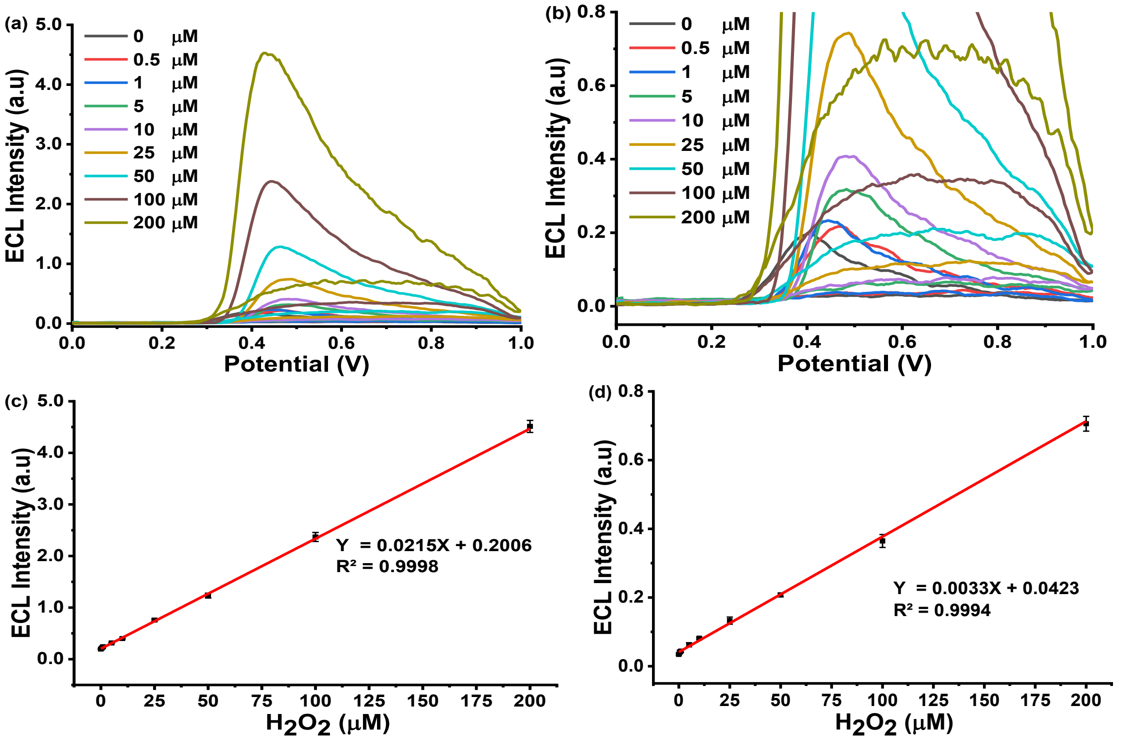

Fig. 4 (a) The ECL signals of luminol containing H_2O_2 concentration levels with; (b) insert The ECL^2 signals. The calibration curve of; (c) the ECL^1; (d) ECL^2 signal on SPGE

The analytical performance in terms of repeatability, reproducibility, and stability was assessed based on ECL signals of luminol in the presence of 50 μM H_2O_2, measured 7 times for repeatability, 4 times on different days for reproducibility, and 3 times in the 4th week for stability. The ECL Signals for H_2O_2 detection (Figure 5a) and the corresponding intensity plots (Figure 5b) for repeatability measurement on SPGE yielded a relative standard deviation (%RSD) of 6.03%, which falls within the acceptance criterion of < ⅔ CV Horwitz of 9.85% indicating good repeatability. The excellent reproducibility of H_2O_2 detection was confirmed with an RSD of 3.41% (Figure 5c), evaluated using a two-sample t-test comparing the ECL intensity on different days. The calculated t-value was 0.139, with a p-value of 0.892, compared with t-table value (0.05, 6) of 2.447. Since t-value ≤ t-table, the results confirmed no significant difference between measurements, demonstrating excellent reproducibility (Faridah et al., 2020). The stability of H_2O_2 detection over different weeks showed a %RSD of 9.74% (Figure 5d). t-value of stability was lower than t-table value (0.05, 4) of 2.776 as shown in Table 2, indicating no significant difference over time.

Table 2. The t-test of reproducibility on different days and stability in 4th week for H_2O_2 detection

Performance	Reproducibility	Stability			
		1st week	2nd week	3rd week	4th week
t-value	0.139	Control	1.686	0.366	1.435
t-table	2.447		2.776	2.776	2.776
p-value	0.892		0.103	0.518	0.152

Term of acceptance: p-value > (α = 0.05) and t-value < t-table (α = 0.05)

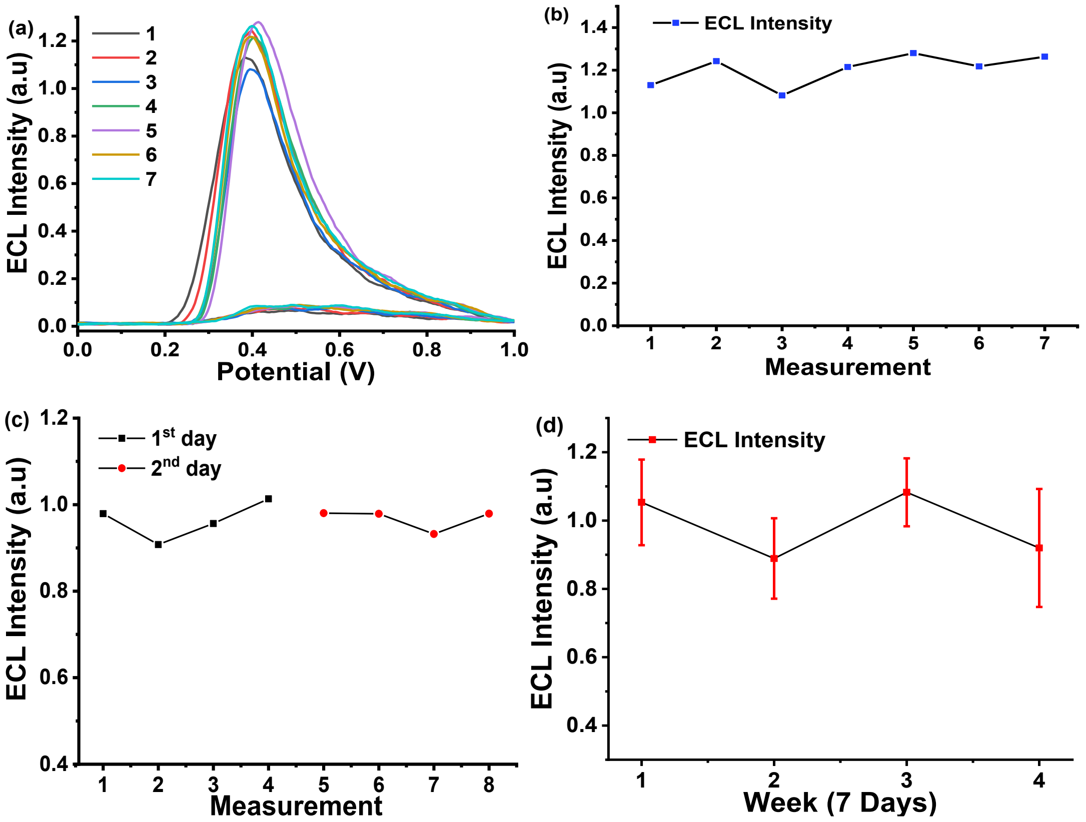

Fig. 5 (a) The ECL signals of luminol in containing of H_2O_2 with; (b) plot The ECL signals for repeatability; (c) reproducibility; (d) stability

The selectivity of ECL measurement for H_2O_2 detection on SPGE was evaluated using 1 mM luminol and 50 μM H_2O_2 solution in 0.1M PBS (pH9) with potential interference from Cl^-, NH_4^+, CO_3^{2-}, SO_4^{2-} and glucose at 100 μM, measured 3 times (Figure 6a). The results demonstrated good selectivity in the presence of Cl^- and NH_4^+ (Figure 6b), while CO_3^{2-} and SO_4^{2-}significantly decreased the ECL intensity. Glucose increased ECL intensity due to the interaction of its hemiacetal group with $Au(OH)_3$, forming D-gluconate radical, which could react with luminol anions (Opallo & Dolinska, 2018). CO_3^{2-} serves as a scavenger in H_2O_2 systems, interfering with luminol reaction and quenching radicals from H_2O_2. SO_4^{2-} react with radicals, generating sulfate radicals ($SO_4^{\bullet -}$), which trigger a propagation reaction (Syukur et al., 2023). This process reduces available $O_2^{\bullet -}$ and disrupts the ECL reaction, which leads to a decline in ECL intensity and reduces sensor efficiency. To minimize interference, CO_3^{2-} and SO_4^{2-} concentrations should be controlled at ≤100 μM .

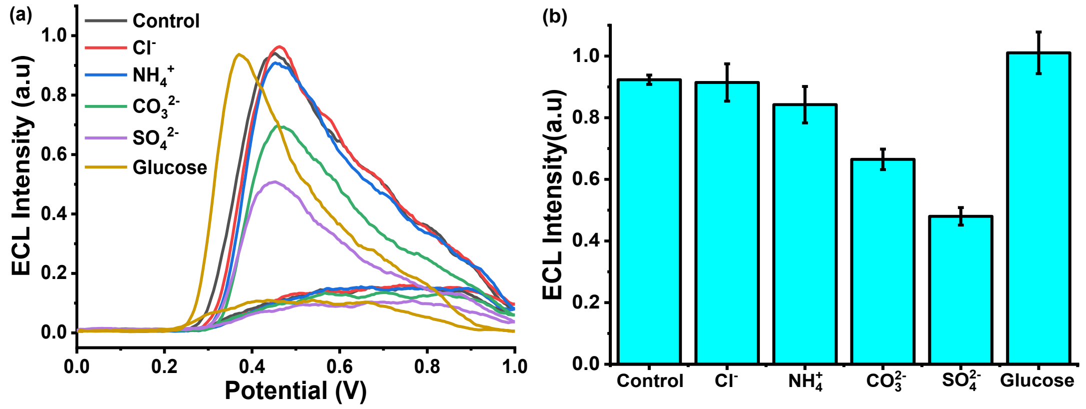

Fig. 6 (a) The ECL signals of luminol in containing of H_2O_2 with several interferences together with; (b) plot average of measurement

Milk and tap water samples were collected and spiked with H_2O_2 following the preparation and methods described previous study (Syukur et al., 2023). The ECL method was applied for H_2O_2 detection in milk and tap water using SPGE. Samples were spiked with H_2O_2 at concentrations of 50, 75, and 100 μM. To validate the method, a SPCE from Metrohm (DRP – 11L type) was used as a comparison (Syukur et al., 2023). The accuracy of spiked samples was determined by calculating the percent recovery, with an acceptable range of 80 – 110%, as required by the Association of Official Analytical Chemists (AOAC) standard. The H_2O_2 recovery on SPGE showed good value, ranging from 83.83 to 106.01%, indicating reliable recovery. Table 3 presents a comparison of H_2O_2 recovery between SPGE and SPCE.

Table 3. The recovery of H_2O_2 spiking in milk and tap water samples on SPGE and SPCE

Electrode	Spike (μM)	Tap water		Milk	
		Found (μM)	Recovery (%)	Found (μM)	Recovery (%)
SPGE	50	42.08 ± 8.60	84.16 ± 17.19	47.83 ± 3.88	95.67 ± 7.65
(DRP-250AT)	75	79.51 ± 5.36	106.01 ± 7.15	71.98 ± 2.89	95.97 ± 3.85
	100	89.63 ± 6.48	89.63 ± 6.48	83.83 ± 1.74	83.83 ± 1.74
SPCE	50	45.74 ± 4.96	91.48 ± 9.92	48.70 ± 4.59	97.41 ± 9.18
(DRP–11L)	75	79.02 ± 1.06	105.35 ± 1.41	78.39 ± 3.75	104.52 ± 5.00
	100	93.91 ± 3.55	93.91 ± 3.55	96.12 ± 3.33	96.12 ± 3.33

4. Conclusions

The determination of H_2O_2 using ECL method on SPGE was successfully achieved, demonstrating high sensitivity and reliability. The ECL^1 intensity was selected as a detection signal, showing a strong linear relationship ($R^2 = 0.9998$) with increasing H_2O_2 concentrations, as represented by the calibration equation $y = 0.0215[H_2O_2] + 0.2006$. The method achieved a LOD of 3.06 μM, a LOQ of 10.20 μM, and a sensitivity of 17.14 a.u μM^{-1} dm^{-2}, indicating excellent analytical performance. The method also exhibited good repeatability, with %RSD ≤ ⅔ CV Horwitz. High reproducibility and stability as confirmed by a t-value ≤ t-table, indicating no significant variations across measurements. These results confirm the robustness and reliability of the proposed ECL-based SPGE sensor for H_2O_2 detection. Consequently, the method offers several advantages, including fast detection, simple operation, and high applicability for real sample analysis. Its effectiveness was validated in milk and tap water, with recovery rates ranging from 83.83 to 106.01%, meeting acceptable analytical standards. As this study represents a preliminary investigation, future research should focus on further optimization of sensor performance, long-term stability assessments, and validation with a wider range of real samples. Additionally, exploring potential miniaturization and integration into portable sensing devices could enhance the practical application of SPGE-based ECL sensors for on-site H_2O_2 monitoring in environmental and food safety applications.

Acknowledgement

The authors gratefully acknowledge to Bioelectrochemistry Research Group Universitas Indonesia which had supported an author. We also extend our appreciation to the editors and reviewers for their constructive feedback, which has significantly enhanced the quality of this work.

Author Contribution

Conceptualization, J.M.S., I.R.; Methodology, A.R.S., J.M.S., M.R.; Validation, A.R.S, I.R.; Formal Analysis, J.M.S.; Investigation, J.M.S; Resources, J.M.S., M.R.; Data Curation, I.R., A.R.S.; Writing–Original Draft Preparation, J.M.S; Writing–Review & Editing, I.R., A.R.S.; Visualization, J.M.S., A.R.S.; Supervision, M.R.

Funding

This research was fully financial support by the ministry of education, culture, research and technology of republic Indonesia (Grant No: NKB-986/UN2.RST/ HKP.05.00/2022).

Ethical Review Board Statement

Not available.

Informed Consent Statement

Not available.

Data Availability Statement

Not available.

Conflicts of Interest

The authors declare no conflict of interest.

Open Access

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Biographies of Authors

Junjunan Muhammad Syukur, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, West Java 16424, Indonesia.

- Email: junjunan.muhammad@sci.ui.ac.id
- ORCID: N/A
- Web of Science ResearcherID: N/A
- Scopus Author ID: N/A
- Homepage: N/A

Afiten Rahmin Sanjaya, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, West Java 16424, Indonesia.

- Email: afiten.rahmin@ui.ac.id
- ORCID: 0000-0001-6834-4588
- Web of Science ResearcherID: ISB-6405-2023
- Scopus Author ID: 58117944600
- Homepage: N/A

Isnaini Rahmawati, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, West Java 16424, Indonesia.

- Email: isnaini.rahmawati@sci.ui.ac.id
- ORCID: 0000-0003-1327-592X
- Web of Science ResearcherID: N/A
- Scopus Author ID: 57194336516
- Homepage: N/A

Muhammad Ridwan, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, West Java 16424, Indonesia.

- Email: muhammaad.ridwan@sci.ui.ac.id
- ORCID: 0000-0002-0201-2684
- Web of Science ResearcherID: JFB-1258-2023
- Scopus Author ID: 55755104000
- Homepage: N/A