

Modification of Screen-printed Carbon Electrodes with Gold Particles to Enhance Luminol Electrochemiluminescence for Hydrogen Peroxide Detection

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Screen-printed carbon electrodes (SPCEs) were modified with gold particles to improve luminol electrochemiluminescence (ECL) in the presence of H₂O₂ as the co-reactant. Gold particles with sizes ranging from 10 to 50 nm were successfully deposited by the electrochemical technique with around 3.4% (w/w) gold on the carbon surface. The gold-modified SPCE (Au-SPCE) increases the ECL intensity of luminol around 2.5 times more than that using the unmodified SPCE. These signals were also found to be linearly correlated to H₂O₂ concentration. At an optimum pH of 9, the ECL signals of 1 mM luminol in 0.1 M phosphate buffer solution using the Au-SPCE are linear in the H₂O₂ concentration range from 0.5 to 200 μM ($R^2 = 0.99$) with an estimated detection limit of 4.78 μM. The developed sensor also showed the excellent repeatability and reproducibility of the ECL signals. Moreover, H₂O₂ detection in milk and tap water samples was also successfully demonstrated, indicating that the developed sensor is promising for H₂O₂ detection applications.

1. Introduction

Electrochemiluminescence (ECL) is the luminescence produced and controlled by an electrochemical reaction, which undergoes a speciation reaction and a high-energy electron transfer reaction to produce an excited state that can emit light on an electrode surface.^(1–3) The ECL method is widely used in chemical analysis, food testing, clinical diagnostics, immunoassays, and biosensing as well as in environmental protection industries owing to its advantages, such as easy operation, low cost, and fast and accurate measurement.⁽²⁾

Two pathways of ECL are widely known: annihilation and co-reactant.⁽⁴⁾ In the annihilation pathway, the oxidation reaction of luminophores occurs to produce an excited state that can emit

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light as a result of an exergonic electron transfer between species of radical ions on the surface of an electrode.^(5,6) This pathway involves highly reactive intermediates and usually requires pure aprotic solvents and deoxygenated solution conditions, making it difficult to use in analytical applications.⁽²⁾ On the other hand, the co-reactant ECL pathway uses luminophores in the presence of an electroactive co-reactant. The co-reactant is oxidized or reduced on the electrode surface when a potential is applied, producing intermediate species that react with luminophores to promote it to an excited state. This pathway is established in the ECL analytical instruments available commercially.⁽⁶⁾ Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), $\text{Ru}(\text{bpy})_3^{2+}$, or quantum dots are generally used as luminophores, whereas H_2O_2 , tri-*n*-propylamine (TPrA), and $\text{S}_2\text{O}_8^{2-}$ were reported to be successfully used as co-reactants.^(1,3,4,7)

Screen-printed carbon electrodes (SPCEs) have been commonly used in electrochemical sensors. SPCE in a point-of-care testing system leads to a portable electrochemical detection instrument with the advantages of disposable electrode, low sample volume, fast detection method, and low cost.⁽⁸⁾ Moreover, gold particles are of particular interest owing to their high surface area, excellent conductivity, and catalytic properties.^(9,10) The modification of SPCE using gold particles was reported to increase the ECL intensity of tris (hydroxymethyl) aminomethane in the presence of O_2 as a co-reactant.⁽⁹⁾ Dispersing gold particles in mesoporous silica films is reported to enhance the ECL of luminol.⁽¹¹⁾ However, studies on the effects of the modifications of SPCE with gold particles on the ECL intensity are still lacking, particularly for analytical purposes.

In this research, the co-reactant ECL pathway with luminol as the luminophore and H_2O_2 as the co-reactant was investigated using the gold-modified SPCE (Au-SPCE). H_2O_2 oxidizes luminol to produce an excited species of 3-aminophthalate that can emit ECL light.⁽¹²⁾ The linear correlation of the ECL signals to the H_2O_2 concentration is promising for sensor and biosensor applications.

H_2O_2 is an essential compound used in many industries.⁽¹³⁾ It plays a crucial role in biological systems as its accumulation can damage lipids, proteins, and DNA.⁽¹⁴⁾ Moreover, H_2O_2 is a chemical compound for water treatment and used for the disinfection and sterilization of milk products. Therefore, it is important to measure residual H_2O_2 in tap water and milk to control H_2O_2 requirement.⁽¹⁵⁾

Various methods have been developed to detect H_2O_2 , such as titration,⁽¹⁶⁾ spectrophotometry,⁽¹⁷⁾ fluorescence,⁽¹⁸⁾ colorimetry,⁽¹⁹⁾ and flow injection analysis combined with chemiluminescence.^(13,20) Nevertheless, although all these methods can reliably and successfully detect H_2O_2 , they have disadvantages such as low sensitivity, expensive operation, and high maintenance costs, as well as long-time measurement and preparation.⁽²¹⁾

The modification of SPCE with gold particles enhances the ECL signals of luminol around 2.5 times compared with when using the unmodified SPCE. The signals were found to be increased around 10 more times in the presence of H_2O_2 . These signals have a linear relationship with the H_2O_2 concentration. The application of this system to H_2O_2 detection in water and milk samples was successfully demonstrated, indicating that the modification of SPCE with gold particles is suitable for the development of electrodes that can sensitively and selectively detect H_2O_2 based on the ECL of luminol.

2. Experimental Procedure

2.1 Materials and methods

All reagents were of analytical grade and used without purification. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) was purchased from Tokyo Chemical Industry, whereas potassium ferricyanide ($K_3[Fe(CN)_6]$) was supplied by WAKO (Japan). Tetrachloroauric (III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$), 30% hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), potassium hydroxide (KOH), dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), and all other chemicals were purchased from Sigma-Aldrich. All the solutions were prepared using double-distilled water with a maximum conductivity of $0.056 \mu S/cm$ obtained from the Direct-Q[®]3 UV Remote Water Purification System (Direct-Q3 UV, Millipore).

All the electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT204, Metrohm), connected to a computer using Nova 2.1 software and a DropSens connector (DSC, Metrohm) to connect with the working electrodes [Dropsens SPCE (DRP-11L, Metrohm) and screen-printed gold electrodes (SPGE) (250AT, Metrohm)]. The screen-printed electrodes from carbon consisted of a carbon auxiliary electrode and a Ag/AgCl reference electrode, while SPGE consisted of a platinum auxiliary electrode and a Ag reference electrode also. The ECL intensity was measured using a photomultiplier tube module (PMT, H11902-20, Hamamatsu) placed in a dark box at a height of 2.0 cm from the electrochemical cell.⁽¹⁾

2.2 Preparation of electrodes

The modification of the SPCE surface with gold particles was performed by the chronoamperometry technique with a drop (50 μL) of 60 $\mu g/mL$ $HAuCl_4$ solution.⁽²²⁾ A potential of -0.2 V was applied with various electrodeposition times to obtain the optimum condition. Surface morphological and elemental analyses of the unmodified SPCE, Au-SPCE, and SPGE were performed by field emission scanning electron microscopy (FE-SEM) combined with energy-dispersive X-ray spectroscopy (EDS) (JIB-4610F, JEOL).

2.3 ECL measurements

ECL measurements were performed by dropping 40 μL of 0.1 M phosphate buffer solution (PBS) containing 1.0 mM luminol in the absence and presence of H_2O_2 with a volume ratio of 1:1 on the Au-SPCE surface. Cyclic voltammetry (CV) with a scan rate of 100 mV/s was carried out. The pH of the solution was varied to optimize the measurements. The ECL intensity was measured during the CV measurements by using the PMT at a potential of 800 mV.

The ECL analysis was conducted on tap water and milk samples. The tap water sample was prepared by spiking different amounts of H_2O_2 into a solution of 0.1 mM luminol in 0.1 M PBS (pH 9), whereas the milk sample was treated by adding 0.5 mL of 1.0 M H_2SO_4 solution, then

centrifuged at 10000 rpm for 10 min. The filtrate was diluted by a factor of 20, then H_2O_2 was added at different concentrations.

3. Results and Discussion

3.1 Modification of SPCEs with gold particles

Prior to modification, the electrochemical behavior of the unmodified SPCE was examined by CV as shown in Fig. 1(a). No peak was observed in the voltammogram of the unmodified SPCE in 0.1 M PBS (pH 9) with (red line) and without (black line) H_2O_2 , indicating that H_2O_2 cannot be detected by voltammetry using this electrode. However, when 1 mM luminol was added into the solution of 0.1 M PBS (pH 9) (blue line), an oxidation peak at a potential of 0.3 V (vs Ag/AgCl) was clearly observed. The peak current slightly increased when H_2O_2 was added to the system (green line).

The modification of SPCE with gold particles was performed by electrodeposition at a potential of -0.2 V (vs Ag/AgCl). After the modification [Fig. 1(b)], the voltammograms of 0.1 M PBS (pH 9) in the absence of luminol showed a couple of oxidation–reduction peaks at

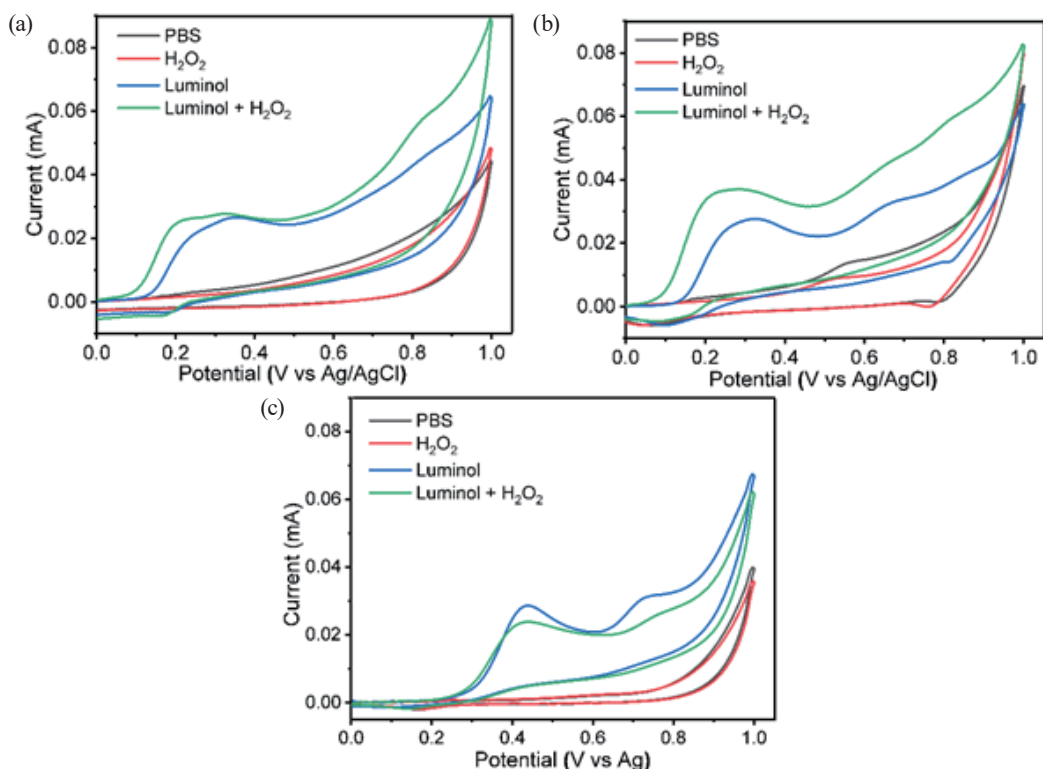


Fig. 1. (Color online) Cyclic voltammograms of 0.1 M PBS (pH 9) (black line) consisting of 1 mM luminol (blue line), $50 \mu\text{M}$ H_2O_2 (red line), and both 1 mM luminol and $50 \mu\text{M}$ H_2O_2 (green line) using (a) unmodified SPCE, (b) Au-SPCE, and (c) SPGE. A scan rate of 100 mV/s was applied.

potentials of +0.5 and +0.8 V (black line), which are characteristic of gold. When luminol was added to the solution, an oxidation peak at around +0.3 V was observed (blue line), indicating that this peak was strongly related to the oxidation of luminol.

Furthermore, the potential of this peak is very close to that obtained using the unmodified SPCE as shown in Fig. 1(a). The slight shift of the peak potential to a more negative side was probably due to the increase in the conductivity of the electrode after the modification with gold particles.^(9,23) Further experiments with the addition of H₂O₂ to the solution of luminol in PBS (pH 9) (green line) showed a slight increase in oxidation peak using the modified SPCE. However, different from SPCEs that are not electroactive to H₂O₂, the gold particles on the electrode surface can oxidize H₂O₂ as shown by the voltammograms of H₂O₂ without luminol, which generates a peak at the potential of around +0.2 V (red line).

The confirmation of luminol oxidation behavior was conducted using SPGE, which showed that the oxidation of luminol occurs around the potential of +0.45 V vs Ag [Fig. 1(c)], which is significantly higher than that obtained using the unmodified SPCE and Au-SPCE. This difference can be explained by the different reference electrodes used in the screen-printed electrodes. Whereas a Ag/AgCl system was used in the SPCE, the reference electrode system in the SPGE was silver (Ag). However, in contrast to those using the unmodified SPCE and Au-SPCE, H₂O₂ decreases the oxidation current of luminol (green line). The oxidation of H₂O₂ seems to disrupt the oxidation of luminol on the SPGE surface. This phenomenon should also occur on the Au-SPCE surface. However, it seems that the limited number of gold particles on the electrode surface causes the low oxidation of H₂O₂.

Cyclic voltammograms and ECL signals of 1 mM luminol in 0.1 M PBS (pH 9) with and without H₂O₂ using SPCEs after gold modification with various deposition times are shown in Fig. 2. In all the voltammograms, a well-defined oxidation peak of luminol at around +0.25 V (vs Ag/AgCl) was observed. Furthermore, similar current responses were obtained in the voltammograms both in the absence and presence of H₂O₂; however, a slight current increase was observed when the gold deposition times increased. This increase is reasonable as the number of gold particles on the carbon surface also increased, and gold is known to have a higher conductivity than carbon.^(23,24)

During voltammetry measurements, ECL signals were also recorded. An ECL signal was generated at a potential the same as the luminol oxidation peak potentials in the voltammograms. In contrast to that observed in the voltammograms, the ECL intensity of luminol oxidation significantly changed with increasing gold deposition time, particularly in the presence of H₂O₂. In fact, in the absence of H₂O₂, the oxidation of luminol in a basic solution also generates ECL as the electrochemical oxidation produces 3-aminophthalate, which then relaxes and emits luminescence.⁽²⁵⁾ In the presence of H₂O₂, the oxidation of H₂O₂ was reported to produce OH• and OH⁻ and increase the intensity of ECL signals by inducing the oxidation of luminol.^(26,27) Accordingly, the presence of H₂O₂ increased the intensity of ECL signals around 10 times.

The dependence of the luminol ECL signal-to-background (S/B) ratio on the gold deposition time is shown in Fig. 2. The data was extracted from the peak at 0.3 V without H₂O₂ as the background peak with H₂O₂ as the signal. The increase in the number of gold particles on the carbon surface increased not only the ECL intensity but also the background current. The

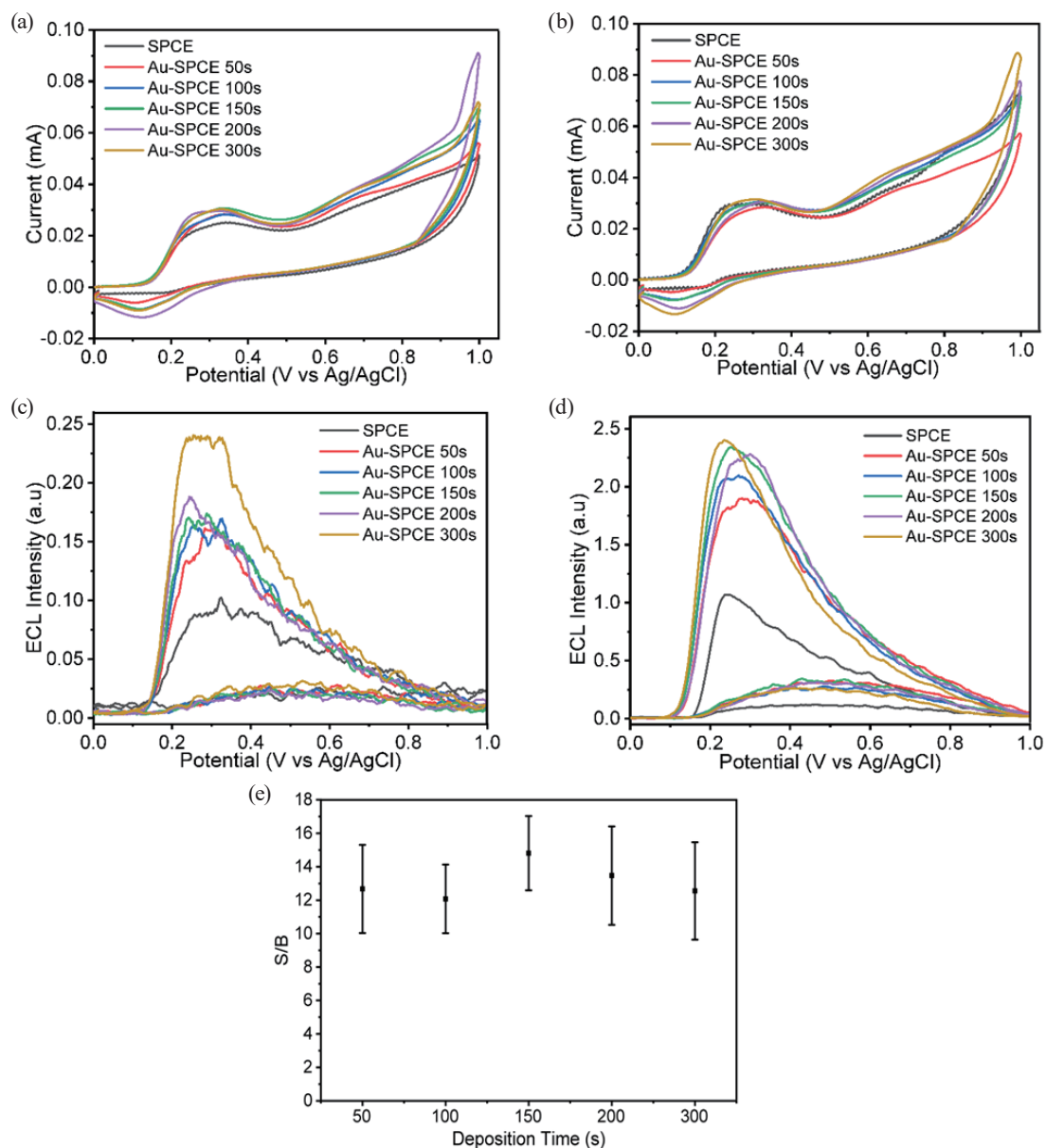


Fig. 2. (Color online) (a) Cyclic voltammograms and (b) ECL signals of 1 mM luminol in 0.1 M PBS (pH 9) in the absence of H_2O_2 ; (c) cyclic voltammograms and (d) ECL signals of 1 mM luminol in 0.1 M PBS (pH 9) in the presence of $50 \mu M H_2O_2$ using Au-SPCE with various gold deposition time, and (e) the dependence of the S/B ratio on the deposition time of gold.

highest S/B ratio was found at the deposition time of 150 s, in which the intensity of the ECL signal using this modified SPCE increased around 2.5 times compared with that observed before the modification. Therefore, this deposition time was selected for the preparation of the modified SPCE.

Further examination of Au-SPCE by surface characterization using FE-SEM compared with the unmodified SPCE [Fig. 3(a)] indicated that gold particles were successfully deposited, as

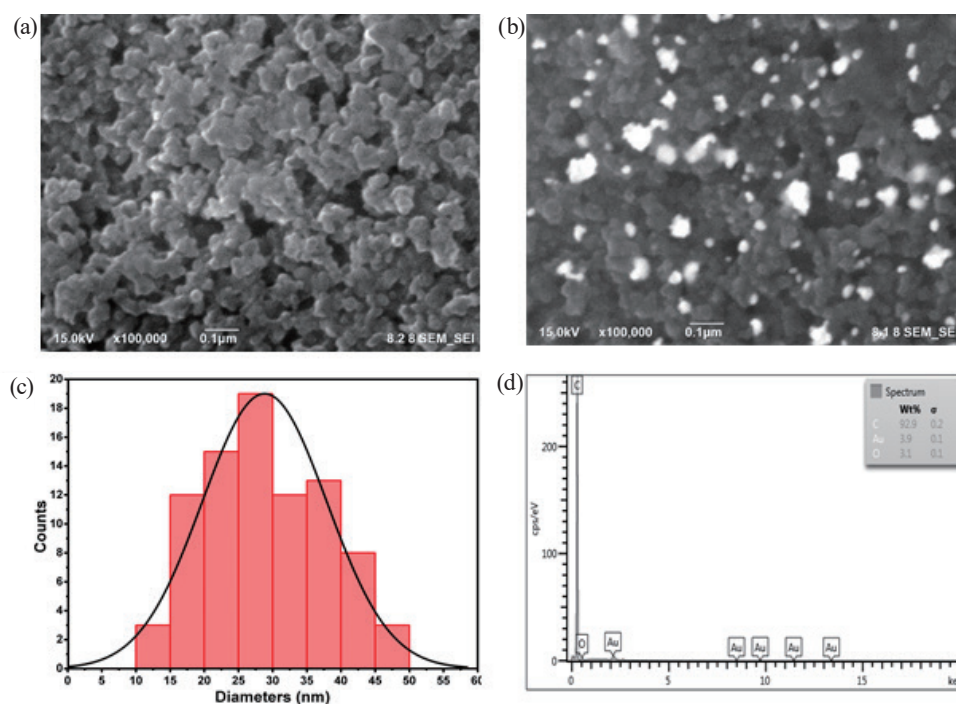


Fig. 3. (Color online) Typical SEM image of (a) unmodified SPCE and (b) Au-SPCE together with its (b) gold particle size distribution calculated with Ime-J software. (d) EDX spectra.

shown by the homogeneous distribution of white dots on the carbon surface as shown in Fig. 3(b). Particles with sizes ranging from 10 to 50 nm were observed [Fig. 3(c)], where the elemental analysis of Au-SPCE performed by EDX as shown in Fig. 3(d) revealed that the average percentage of the deposited gold was 3.9%.

Furthermore, the active surface areas of the electrodes were evaluated by CV of 1 mM $K_3[Fe(CN)_6]$ in 0.1 M PBS (pH 9) with various scan rates ranging from 25 to 200 mV/s (Fig. S1). The Randles–Sevcik equation (Eq. 1) was applied to determine the active surface areas:

$$i_p = 2.687 \times 10^5 n^{3/2} A D^{1/2} C v^{1/2}, \quad (1)$$

where i_p is the anodic peak current, n is the number of electrons transferred, D is the diffusion coefficient ($7.6 \times 10^6 \text{ cm}^2 \text{ s}^{-1}$), C is the concentration of $K_3[Fe(CN)_6]$, v is the scan rate, and A is the active surface areas.⁽²⁴⁾ The estimated active surface area of Au-SPCE was 0.1119 cm^2 , which is significantly larger than that before the modification (0.0607 cm^2). This result is confirmed by the higher intensity of ECL signals using the modified SPCE than that using the unmodified one [Figs. S1(d) and S1(c), respectively]. In fact, the gold particles have the potential to decrease the intensity of the ECL signals of luminol– H_2O_2 , as the direct oxidation of H_2O_2 at the gold sites can decrease the amount of H_2O_2 which can oxidize luminol. Therefore, it is important to control the number of gold particles on the SPCE surface.

3.2 ECL measurements

The applied potential range of ECL measurements was studied to optimize the measurement condition. The ECL signals of 1 mM luminol in 0.1 M PBS (pH 9) were obtained in various potential ranges in the absence [Fig. S2(a)] and presence [Fig. S2(b)] of H_2O_2 . In the potential range from 0 to +1.0 V, a single peak at around +0.3 V was observed with and without H_2O_2 . This peak confirms the previous results generated from the ECL emission of the excited species of 3-aminophthalate.^(25,28) Furthermore, shifting the starting potential to be more negative continuously increases this ECL peak.

In addition to the ECL peak at +0.3 V, another peak seems to appear at a more negative potential when the starting potential shifted to -0.2 V. This peak becomes clearer in the wider potential ranges. Applying a very wide potential range such as from -1.0 to +1.0 V confirms the appearance of another ECL peak at around -0.75 V. This peak has a higher intensity than the ECL peak at around +0.3 V. This ECL signal was reported to be induced by the electrochemical reduction of water to form radicals, which then reacted with luminol to form the excited species.^(25,28) The radical formation at the negative potential seems to significantly increase the ECL intensity of luminol at the potential of +0.3 V.

In the presence of H_2O_2 , the peak at +0.3 V increased when the potential ranging from 0 to +1.0 V was applied as discussed above. However, when a wider potential was applied, the interference of the ECL signals at -0.75 V decreased the intensity of the ECL signals at +0.3 V.⁽²⁸⁾ The probable reason is the direct reaction between H_2O_2 and the radicals from water electrolysis, which affects the decrease in the rate of luminol oxidation. To find the optimum applied potential range, the ECL intensity in the absence of H_2O_2 was employed as the background, whereas that in the presence of H_2O_2 was employed as the signal. The dependence of the ECL S/B ratio on the potential range is shown in Fig. 4(a). This figure shows that the highest S/B ratio was obtained when the potential range from 0 to +1.0 V was applied. Accordingly, this potential range was selected for the next experiments.

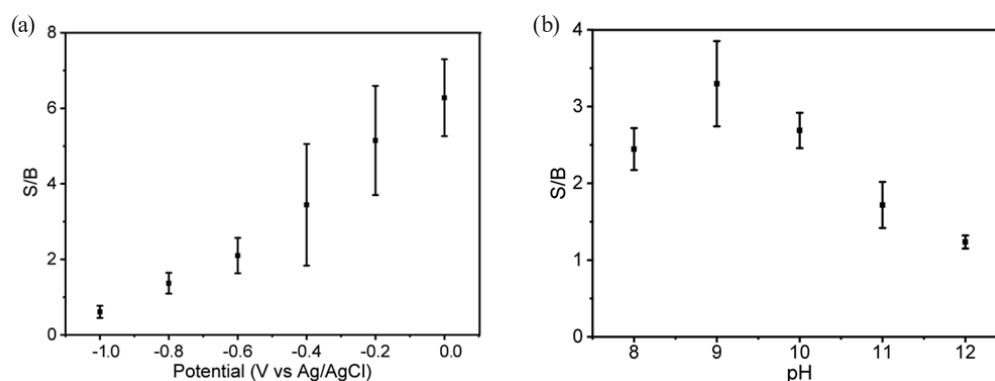


Fig. 4. (Color online) Dependences of the S/B ratio on the (a) starting applied potential and (b) solution pH.

The ECL signals of luminol at various pHs using Au-SPCE were studied in the absence and presence of H_2O_2 [Figs. S3(a) and S3(b), respectively]. PBS, prepared from 0.1 M K_2HPO_4 adjusted with 0.1 M KOH to produce pHs 9 to 12, was used as the buffer. The use of more suitable buffer solutions, such as carbonate and ammonium chloride buffers for luminol ECL, was also reported.^(3,29) However, for H_2O_2 detection in food products, PBS was selected. With or without H_2O_2 , the increase in pH from 8 to 12 increased the ECL intensity. In the presence of H_2O_2 , a significant increase in ECL intensity occurred, although H_2O_2 was reported to decompose under the basic condition.^(2,3) The summary of S/B ratios shown in Fig. 4(b) reveals that pH 9 is the optimum pH with the highest S/B ratio of 3.30. Therefore, pH 9 was selected for the optimum pH. The high error bars observed in both experiments with the various starting potentials and pHs [(Fig. 4(a) and Fig. 4(b), respectively)] were probably due to the effect of water electrolysis, resulting in the instability of the ECL signals. Initiating the potential at 0.0 V under pH 9 conditions is a suitable strategy to prevent issues arising from water electrolysis.

3.3 Performance of H_2O_2 sensors

Figure 5(a) shows the ECL signals at various concentrations of H_2O_2 from 0.5 to 200 μM in 0.1 M PBS (pH 9) in the presence of 1 mM luminol using Au-SPCE. A linear correlation of the ECL signals with the H_2O_2 concentrations was observed with a linear equation of ECL intensity of $y = 0.0241[\text{H}_2\text{O}_2] + 0.0785$ [Fig. 5(b)]. The sensitivity of 0.0241 a.u. $\mu\text{M}^{-1} \text{cm}^{-2}$ can be

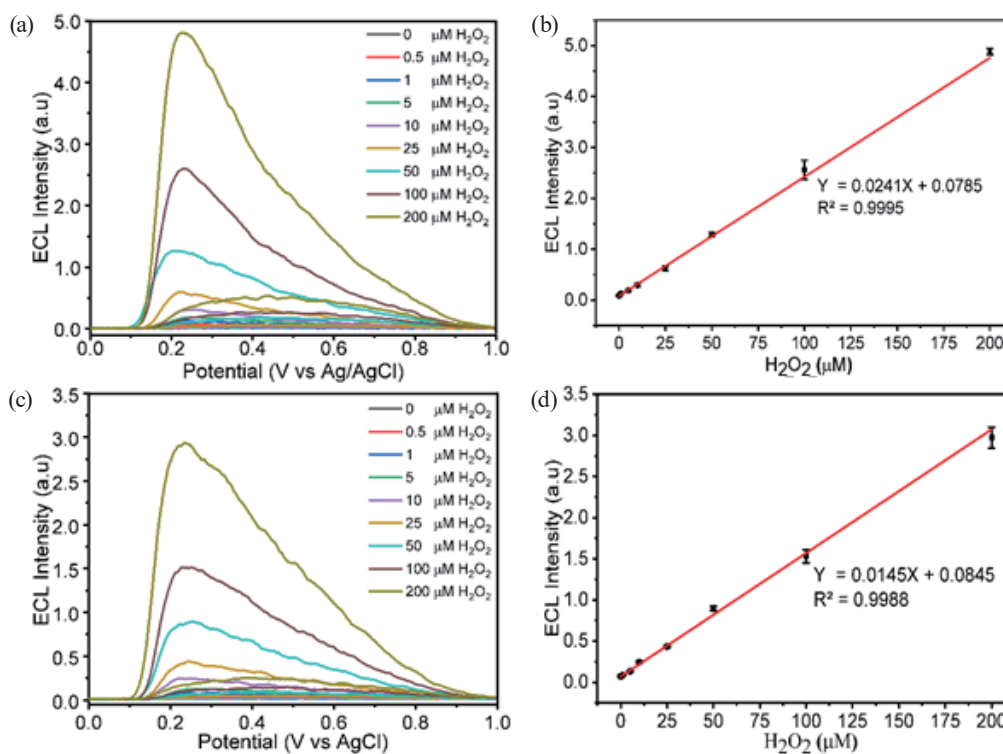


Fig. 5. (Color online) ECL signals of 1 mM luminol in 0.1 M PBS (pH 9) in the presence of various concentrations of H_2O_2 using (a) Au-SPCE and (c) unmodified SPCE together with the correlations of the ECL signals in (b) and (d), respectively. The scan rate was 100 mV/s.

achieved with the limit of detection (LOD) of $4.78 \mu\text{M}$ and the limit of quantification (LOQ) of $15.92 \mu\text{M}$. This result indicated that the SPCE modified with Au particles showed a higher performance than the unmodified SPCE with an LOD of $7.53 \mu\text{M}$, an LOQ of $25.10 \mu\text{M}$, and a sensitivity of $0.0148 \text{ a.u. } \mu\text{M}^{-1}\text{cm}^{-2}$ [Fig. 5(c)]. Furthermore, the sensitivity of the developed sensor was higher with the modified electrode (around 2 times lower LOD) than with the unmodified one [Fig. 5(d)].

The repeatability of the luminol ECL signals in the presence of $50 \mu\text{M H}_2\text{O}_2$ using Au-SPCE was compared with that using SPCE for seven consecutive measurements. An excellent repeatability with a relative standard deviation (RSD) of 2.48% was achieved [Fig. 6(a)]. Good reproducibility of the ECL signals was also achieved in four measurements on the first and second days, showing a reproducibility of 3.05% RSD [Fig. 6(b)]. Furthermore, the electrode stability was also determined for three measurements of the ECL signals on the 4th week with an RSD of 4.52% [Fig. 6(c)]. The analytical performance characteristics of the ECL–luminol sensor for H_2O_2 detection using the unmodified and modified SPCEs are summarized in Table 1.

The performance of the developed H_2O_2 sensor using both electrodes was also found to be comparable to that of the previous sensors as shown in Table 2. AuNPs-MSF/ITO and ITO-PET(SEES) showed a lower LOD possibly owing to the higher surface area; however, the

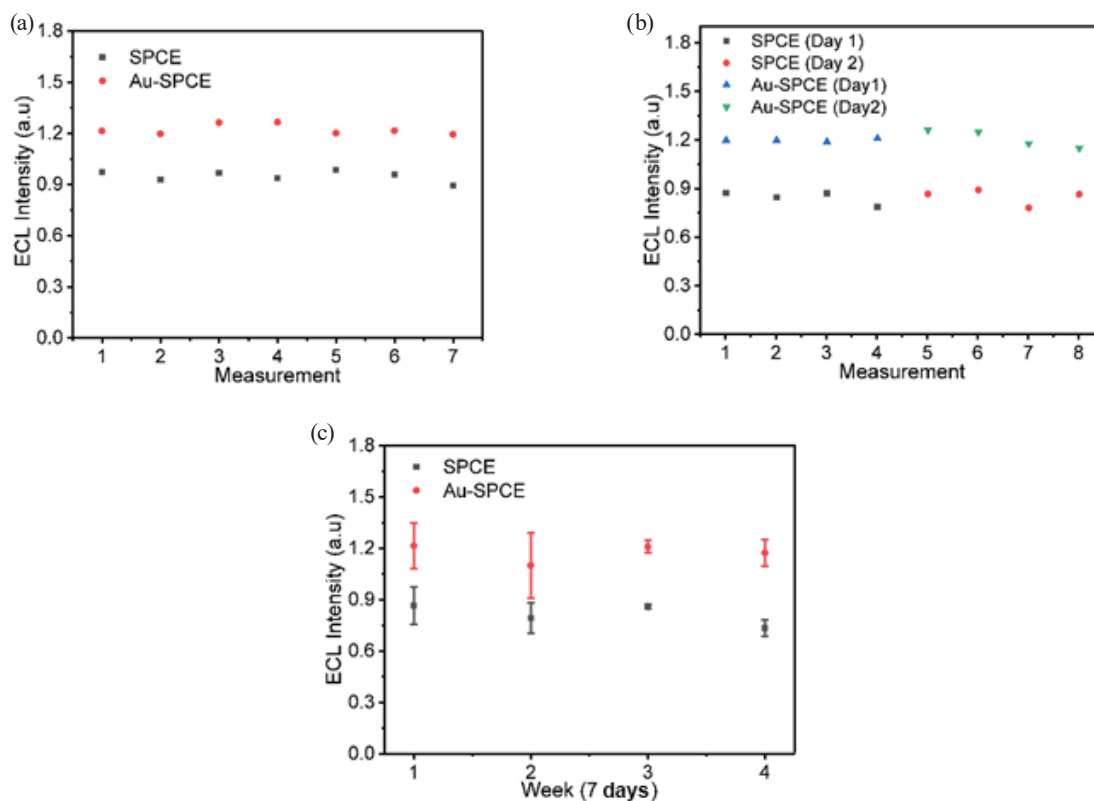


Fig. 6. (Color online) ECL signals of 1 mM luminol in 0.1 M PBS (pH 9) in the presence of $50 \mu\text{M H}_2\text{O}_2$ using SPCE and Au-SPCE determined in (a) 7 consecutive measurements, (b) measurements on different days, and (c) measurements in different weeks.

Table 1
Analytical performance characteristics of H₂O₂-luminol ECL sensor.

Electrodes	Sensitivity (a.u. μM^{-1})	Conc. Range (μM)	LOD (μM)	Repeatability ($n = 7$) %RSD	Reproducibility (2 days) %RSD	Stability (4 weeks) %RSD
SPCE	0.0148	0.5–200	7.53	3.31	4.84	7.60
Au-SPCE	0.0241	0.5–200	4.78	2.48	3.06	4.52

Table 2
Comparison of current work with other studies using various electrodes for H₂O₂-based ECL detection.

Electrodes	Concentration Range (μM)	LOD (μM)	Ref.
AuNPs-MSF/ITO	0.1–200	0.025	(11)
ITO-PET(SEES)	1–100	0.27	(30)
Paper fluidic closed bipolar electrode	75–500	41	(31)
Cloth-based microfluidic device	25–2500	24	(32)
SPCE	0.5–200	7.53	This work
Au-SPCE	0.5–200	4.78	

modification of both electrodes was expected to be more complex than the proposed sensor using Au-SPCE.

The selectivity of the proposed sensor using both electrodes was investigated by performing an interference study in the presence of possible interferences. Figure 7 shows that both electrodes displayed similar trends towards various interferences. Cl^- and NH_4^+ ions as well as glucose were found to not interfere with the ECL signals of luminol for H₂O₂ detection with no significant change in ECL response. However, CO_3^{2-} is likely to slightly interfere with the proposed sensor by decreasing the ECL intensity. Carbonate is well recognized as a scavenger in H₂O₂ systems, causing competition between CO_3^{2-} and luminol in the reaction with H₂O₂, thereby decreasing the ECL intensity of luminol.⁽⁶⁾ Furthermore, SO_4^{2-} was found to significantly interfere with the ECL signals of luminol as it was reported that when SO_4^{2-} reacts with $\bullet\text{OH}$ radicals, a propagation reaction occurs, decreasing the amount of reaction between $\bullet\text{OH}$ and luminol to generate 3-aminophthalate, thereby reducing the ECL intensity.⁽³³⁾ Accordingly, prior to measurement, the interferences of sulphate and carbonate anions should be removed. A precipitation technique using BaOH reagent could be used to overcome this problem; hence, selective detection could be achieved.

The developed sensor was used for H₂O₂ detection in actual samples of milk and tap water. Tap water (1.5 L) was collected on October 18, 2022 from the laboratory of the Department of Chemistry, Universitas Indonesia in Depok, Jakarta, Indonesia. Without any pre-treatment step, except a filtering process to remove solid impurities, a volume of 10 μL was used for ECL measurements. The sample pH was 6.0 and the temperature was 27°C. A chemical analysis of the sample indicated that it contains <0.2 mg of Fe, Cr, Mn, and Hg metal ions per liter. In addition, 0.14 mg L⁻¹ F, 0.55 mg L⁻¹ Zn, 0.8 mg L⁻¹ NO₃⁻, <0.008 mg L⁻¹ NO₂⁻, 0.01 mg L⁻¹ CN, <7 mg L⁻¹ SO₄, 23.27 mg L⁻¹ MnO₄²⁻, and 0.8 mg L⁻¹ free chlorine were also detected. In the case of the milk sample, total fat was 8 g L⁻¹, cholesterol was 25 mg L⁻¹, protein was 8 g L⁻¹, and sodium was 130 mg L⁻¹. Additionally, vitamins A, D, and K, B complex, biotin, choline, zinc, and phosphor were also detected in the sample.

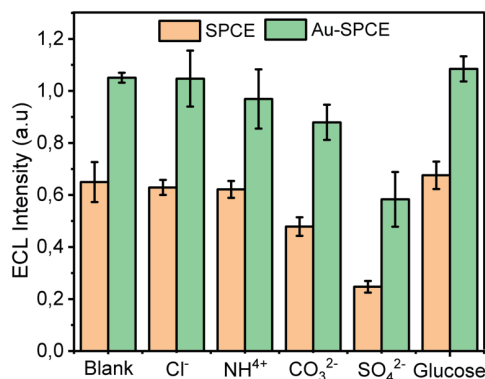


Fig. 7. (Color online) ECL signals of 1 mM luminol in 0.1 M PBS (pH 9) in the presence of 50 μM H_2O_2 using SPCE and Au-SPCE in the presence of possible interferences. The error bar represents the standard deviation from three independent measurements.

Table 3

Determination of H_2O_2 concentration in actual samples of milk and tap water.

Electrode	Spike (μM)	Milk		Tap water	
		Found (μM)	Recovery (%)	Found (μM)	Recovery (%)
Au-SPCE	50	51.93 ± 2.82	103.86	47.62 ± 2.36	95.24
	75	74.98 ± 10.34	99.97	68.72 ± 2.78	91.63
	100	92.48 ± 3.46	92.48	86.11 ± 1.86	86.11

The determination of recovery in the samples was carried out by spiking different amounts of H_2O_2 into the tap water and milk samples with final concentrations of 50, 75, and 100 μM . Table 3 shows the recovery percentages for the determination of H_2O_2 in the spiked samples of milk and tap water. Good recovery (from 86 to 103.86%) of H_2O_2 in milk and tap water was achieved, indicating that the developed sensor is applicable for the detection of H_2O_2 in milk and tap water.

4. Conclusions

A H_2O_2 sensor based on luminol ECL was successfully developed by using commercially available SPCEs modified with gold particles. The estimated electrochemical surface-active area of the electrode was found to be around 0.1119 cm^2 . The presence of gold particles in the SPCE enhanced the ECL signals of luminol around 2.5 times that when using the unmodified one. At the optimum pH of 9, the scan rate of 100 mV/s, and potentials ranging from 0 V to +1.0 V, the intensity of ECL signals of 1 mM luminol in 0.1 M PBS was linear ($R^2 = 0.99$) to the H_2O_2 concentration with a sensitivity of $0.0242 \text{ a.u. } \mu\text{M}^{-1}\text{cm}^{-2}$ and an estimated LOD of 4.78 μM . Furthermore, the performance of the modified SPCE was higher than that of the unmodified one, indicating that the presence of gold particles enhances the performance of H_2O_2 -luminol ECL sensors.

Author Contributions

JMS, IR, AF, and MR interpreted the data and redesigned the figures. YE and TAI supervised this study, developed the concept of the project, and completed the manuscript. All the authors wrote, contributed, and approved the final manuscript.

Conflicts of Interest

None declared.

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Supplements

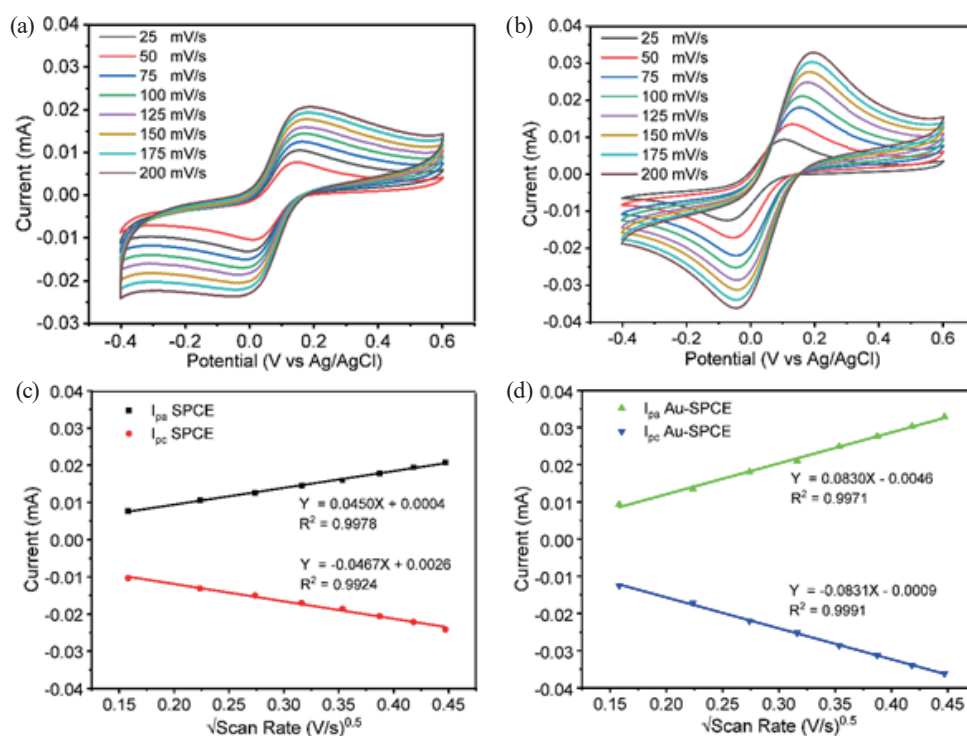


Fig. S1. (Color online) Cyclic voltammograms of (a) SPCE and (b) Au-SPCE in 0.1 M PBS (pH 9) containing 1 mM $K_3[Fe(CN)_6]$ taken at various scan rate, and linear curves of (c) SPCE and (d) Au-SPCE.

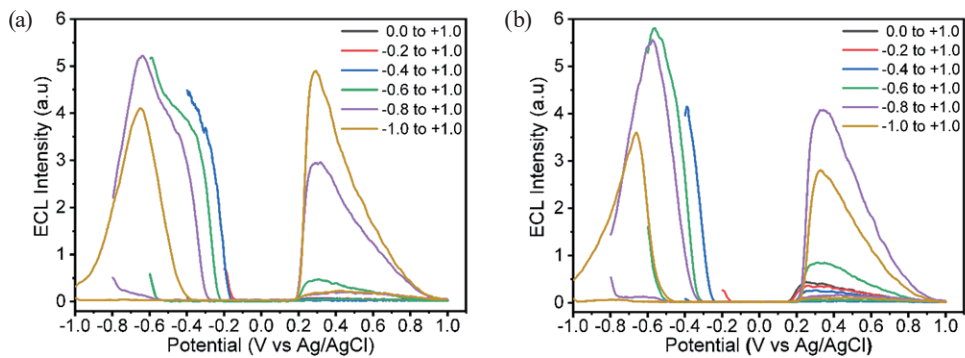


Fig. S2. (Color online) Typical ECL signals of 1 mM luminol in 0.1 M PBS (pH 9) obtained using Au-SPCE in various potential ranges in the (a) absence and (b) presence of $20 \mu M H_2O_2$.

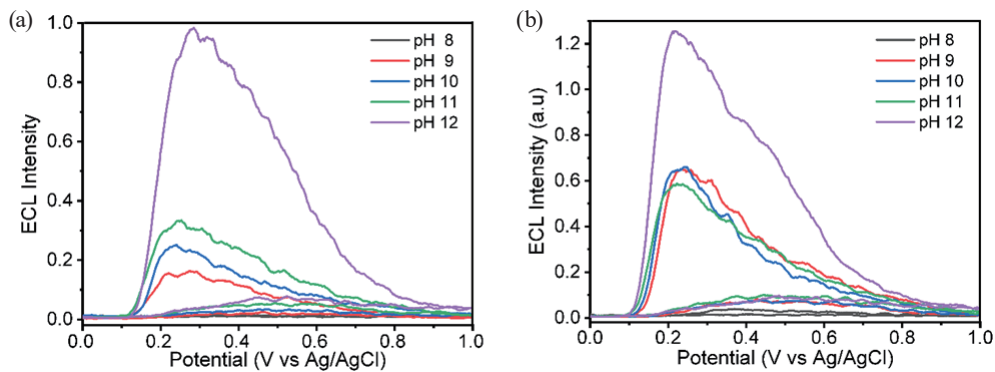


Fig. S3. (Color online) Typical ECL signals of 1 mM luminol in 0.1 M PBS (pH 9) obtained using Au-SPCE at various pHs in the (a) absence and (b) presence of $20 \mu M H_2O_2$.