

Proceedings

# In Situ Generation of Substrate via Bi-Potential Screen-Printed Electrode for Determination of Antioxidant Using Electrochemiluminescence <sup>†</sup>

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**Abstract:** Excess generation of reactive oxygen species (ROS) could lead to diseases such as vasculitis. It is known that antioxidants in beverages suppress the effect of ROS. In this study, we focused on electrochemiluminescence using luminol. It is known that luminescence intensity will increase with increasing amounts of ROS present. We then hypothesized that luminescence will be quenched when antioxidants are also present due to the consumption of ROS by the antioxidants. Application of this system for the detection of ascorbic acid, chlorogenic acid and antioxidants in real samples were demonstrated successfully.

**Keywords:** electrochemiluminescence; antioxidant; screen-printed-electrode; food analysis

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## 1. Introduction

When pathogens such as viruses enter the human body, the immune system generates reactive oxygen species (ROS). However, if ROS are generated in excess, they can attack the body and cause many diseases, including diabetes, high blood pressure and Parkinson's disease [1].

It is known that certain food products such as green tea and citrus fruits have antioxidative properties. A large amount of evidence that antioxidants reduce the incidence of the above mentioned diseases has been reported [2]. Therefore, studying antioxidative properties of food is imperative for human healthcare. However, conventional methods often require long measurement time and large devices or complicated procedures, which render them cumbersome and impractical [3]. Thus, the objective of our research is to develop a fast and simple detection system for antioxidants. To this end, we focused on electrochemiluminescence (ECL) using luminol. ECL has emerged as a powerful analytical tool in a various field such as food analysis and physiological research. ECL has many advantages such as high sensitivity, and reliability. In addition, we adopted disposable screen-printed electrodes (SPEs) because they are easy to mass-produce and cost less compared to conventional electrodes. Utilizing these tools, we aimed to devise an in situ ROS-generating system based on the quenching of luminol ECL by antioxidants on the surface of comb-shaped screen printed bi-potential electrodes [4].

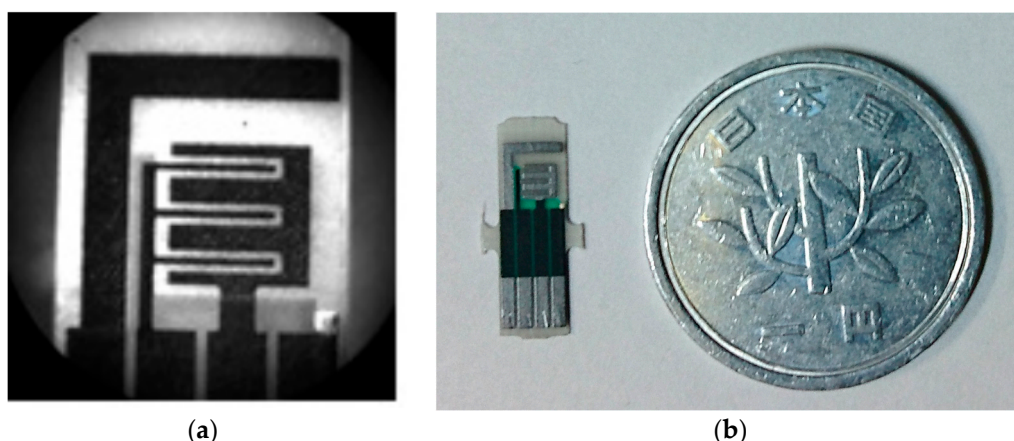
## 2. Materials and Methods

### 2.1. Reagents and Chemicals

Luminol was purchased from Sigma-Aldrich (MO, USA). 200 mM Tris buffer was adjusted to pH8.0 with HCl. 10 mM luminol stock solution was prepared by dissolving in 0.1 M NaOH solution under stirring until dissolved completely and stored at  $-20\text{ }^{\circ}\text{C}$  until use. Trolox and chlorogenic acid were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Beverages (green tea, apple juice) were purchased at a local convenience store. SPEs were purchased from Bio Device Technology, Ltd. (Japan).

### 2.2. Apparatus

ECL imaging was conducted using an image intensifier (model xx2050AH, Photonis, France) and electron multiplying charge coupled device (EM-CCD) camera (ImageEM C9100-13, Hamamatsu photonics K.K., Shizuoka, Japan) mounted on top of an upright microscope (model MVX10, Olympus, Tokyo, Japan). Photons entering the objective lens pass through the image intensifier, which amplifies the resulting light signal. The EM-CCD camera then captures the amplified signal. The ECL intensity was calculated using image analysis software (AquaCosmos Ver2.60, Hamamatsu Photonics K.K., Shizuoka, Japan). Comb-shaped screen-printed electrodes with two working electrodes were used to demonstrate the production of ROS and the oxidation of luminol. The electrodes comprised two working and counter electrodes made of carbon, and an Ag/AgCl reference electrode (Figure 1).

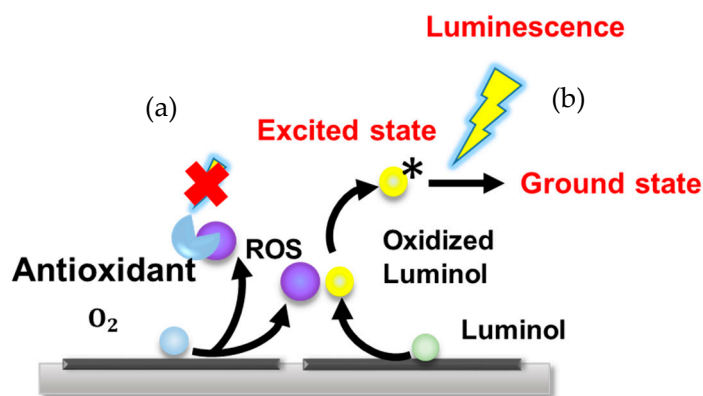


**Figure 1.** (a) Bright field image of 3 comb-shaped screen printed electrode; (b) Size comparison of screen printed electrode with 1 yen coin.

### 2.3. ECL Measurement Procedure

Luminol solution was diluted to  $50\text{ }\mu\text{M}$  in tris buffer, and  $2\text{ }\mu\text{L}$  of this solution was dropped on the electrode surface. Then, the droplet was spread out across the electrode by a round glass slide ( $\phi = 12\text{ mm}$ ).

To conduct the luminescent reaction, a negative voltage was first applied to one of the working electrodes to produce ROS. Next, a positive voltage was applied to the other electrode to oxidize luminol. Then, the oxidized luminol is excited by reacting with ROS, and luminescence is generated by the relaxation of the excited luminol to the ground state (Figure 2). First of all, for the application of positive voltage chronoamperometry (CA,  $500\text{ mV}$ ) and linear sweep voltammetry (LSV, scan rate of  $50\text{ mV/s}$ ,  $0\sim 700\text{ mV}$ ) were compared. In addition, the negative voltage for ROS generation was optimized.



**Figure 2.** The scheme of the principle of the luminol luminescence and quenching by antioxidant (a) Quenching scheme in presence of antioxidant; (b) Scheme of Luminol luminescence with self ROS generating system.

#### 2.4. Antioxidant Detection with Using In Situ ROS Generating Bipotential System

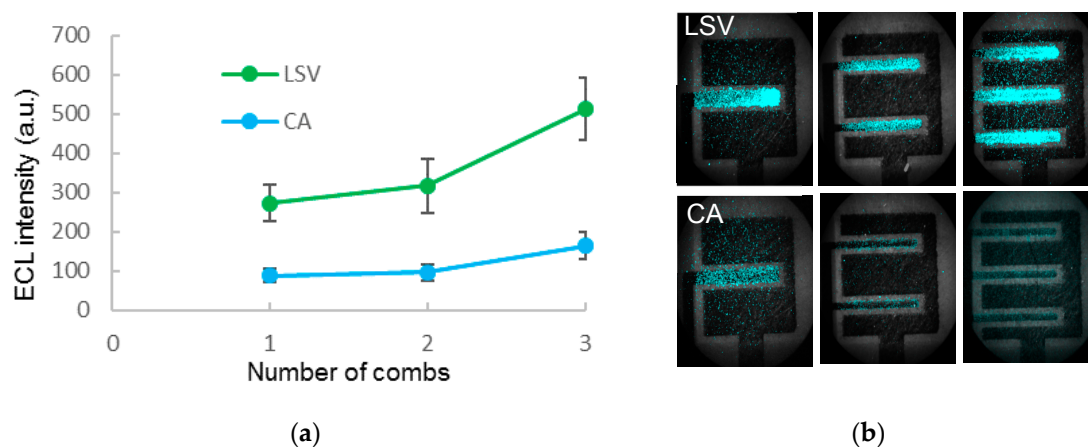
Antioxidants were measured with using the previously described system. A constant voltage of  $-1000$  mV was applied to one of the working electrodes to generate ROS, and a voltage sweep from  $0$  to  $700$  mV was applied to the second electrode for luminol oxidation. In the absence of antioxidants, a strong ECL signal is observed. However, if antioxidants are present in the sample, they deactivate the generated ROS, which decreases the ECL signal.

Samples were mixed with equal volumes of  $100$   $\mu$ M luminol. A range of concentrations ( $0.5$  mM– $2$  mM) of trolox, used as a standard for antioxidant capacity, was also mixed with  $100$   $\mu$ M luminol to create a calibration curve.

### 3. Results and Discussion

#### 3.1. ECL Intensity Measurements Using In Situ ROS Generating Bipotential System

Linear sweep voltammetry was found to be more suitable than chronoamperometry for the imaging of luminescence (Figure 3). The comb-shaped screen-printed electrode was able to detect up to  $10$   $\mu$ M hydrogen peroxide by mixing with  $50$   $\mu$ M of luminol. From these results, it is expected that increasing the number of combs could further enhance the ECL intensity.

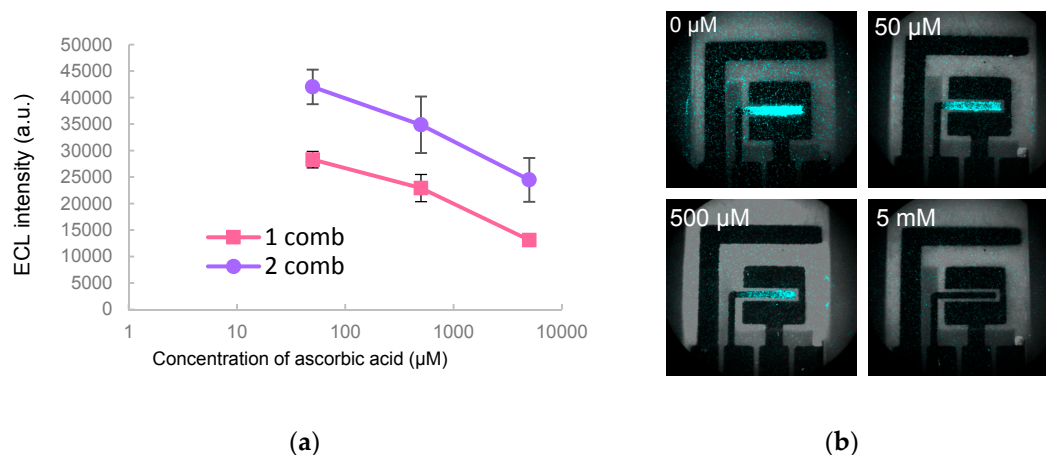


**Figure 3.** (a) ECL intensities of  $50$   $\mu$ M luminol from LSV and CA; (b) Images of luminol ECL on different comb-shaped electrodes by LSV (top) and CA (bottom).

### 3.2. Antioxidant Detection Using In Situ ROS Generating Bipotential System

#### 3.2.1. Measurement of Ascorbic Acid

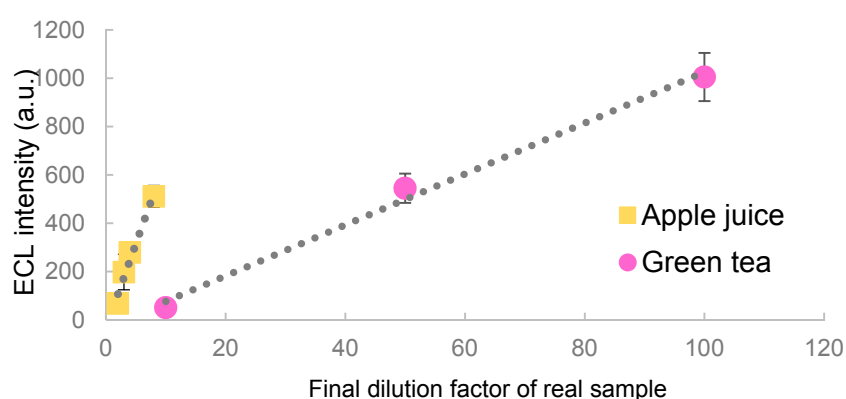
Ascorbic acid was measured through quenching of luminescence induced by in situ generated ROS without external addition of hydrogen peroxide. Quenching was observed in the range from 1 mM to 50  $\mu$ M of ascorbic acid (Figure 4). Quenching effect was also observed by chlorogenic acid.



**Figure 4.** (a) ECL quenching effect of ascorbic acid; (b) Images of the quenching of luminol ECL by different concentrations of ascorbic acid (shown in top left of image).

#### 3.2.2. Real Beverage Samples

In addition, the quenching effects of antioxidants in real beverage samples (Green tea, apple juice etc.) were measured. As the samples were increasingly diluted, recovery of the quenched luminescence was observed (Figure 5). Clear differences were observed among the luminescence quenching from different samples. These results suggest that this system may be used for sensing of other antioxidants through their reactions with ROS.



**Figure 5.** ECL quenching effect of real beverage samples.

## 4. Conclusions

In this work, comb-shaped screen-printed bi-potential electrodes were used for the *in situ* electrochemical generation of ROS. This was then applied for the rapid and facile detection of antioxidants based on the quenching of luminol ECL. This proposed method was successfully used to detect several common antioxidants as well as antioxidant activity in real beverage samples.

**Conflicts of Interest:** The authors declare no conflict of interest.

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