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Remarkable increase in luminol electrochemiluminescence by sequential electroreduction and electrooxidation

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Remarkable increase in luminol electrochemiluminescence by sequential electroreduction and electrooxidation

Xiaoyun Liu,‡a Wenjing Qi,‡a Wenyue Gao,‡a Zhongyuan Liu, a Wei Zhang, a Ying Gao b and Guobao Xu*a

Luminol electrochemiluminescence is dramatically increased by about five hundred times by taking full advantages of both electrochemical reduction and electrochemical oxidation using simple linear sweep voltammetry, leading to sensitive detection.

Electrochemiluminescence (ECL), also called electrogenerated chemiluminescence, is the process that electrogenerated reactants react to produce light.1-4 ECL has superior advantages over conventional chemiluminescence (CL), such as better reproducibility, lower background signal, and easier temporal and spatial control of ECL reactions.5-9 ECL has become a most intensively studied and widely used analytical technique, and has been successfully applied in life science, material science, clinical medicine and environmental monitoring, etc.10-14 Among various ECL systems, luminol ECL is continuously one of most popular systems.14-18 Generally, luminol ECL system can be divided into two main categories, cathodic luminol ECL system and anodic luminol ECL system.19-22 Cathodic luminol ECL system often involves the electrochemical reduction of dissolved oxygen to generate reactive oxygen species (ROS) such as hydrogen peroxide for the trigger of ECL (Eqs 1-3).23-26 Cathodic luminol ECL usually is very weak since luminol cannot be electrochemically oxidized on electrode at negative potentials. Anodic luminol ECL is generally weak without the addition of hydrogen peroxide due to difficult oxidation of water to generate ROS such as hydrogen peroxide, and requires the addition of hydrogen peroxide.

\[ \text{O}_2 + \text{H}_2\text{O} + 2\text{e} \rightarrow \text{ROS} \quad (1) \]

\[ \text{Luminol} + \text{OH}^- \rightarrow \text{Luminol anion} \quad (2) \]

\[ \text{Luminol anion} + \text{ROS} \rightarrow \text{3-Aminophthalate} + h\nu \quad (3) \]

In the present study, we develop a new method to effectively trigger luminol ECL by taking full advantages of both electrochemical reduction and electrochemical oxidation using simple linear sweep voltammetry (Scheme 1). By setting suitable negative initial potentials and positive final potentials in linear sweep voltammetry, ROS is in situ generated effectively through the electrochemical reduction of dissolved oxygen at negative potentials, and then luminol and electrogenerated ROS are oxidized upon scanning potential to sufficiently positive potentials, resulting in hundreds of times increase in anodic luminol ECL intensities.

Fig.1A shows the ECL behaviors of luminol with different initial potentials. The ECL intensity is very weak when the initial potential is 0 V (Fig. 1Aa and 1Ac). In contrast, an intense ECL peak appears at 0.46 V and a very weak ECL peak appear at −0.6 V when the initial potential is −0.8 V (Fig. 1Aa). By comparison, the anodic ECL peak intensity obtained using the initial potential of −0.8 V is about 470 times of that obtained using the initial potential of 0.0 V and is about 150 times of the cathodic ECL peak intensity obtained using the initial potential of −0.8 V. It indicates that the approach taking full advantages of both electrochemical reduction and electrochemical oxidation is much more effective that that using either only electrochemical reduction or only electrochemical oxidation. Fig.1B shows the corresponding cyclic voltammograms (CVs). Only a small electrochemical oxidation peak of luminol appears at about 0.45 V when the initial potential is 0 V, while a strong electrochemical reduction peak of dissolved oxygen appears at −0.67 V besides luminol oxidation peak when the initial potential is −0.8 V. Moreover, the anodic ECL peak intensity obtained using the initial potential of −0.8 V decreases significantly if oxygen is removed (Fig. 1Ad). This result suggests that the generation of ROS by electrochemical reduction of dissolved oxygen results in striking increase in ECL intensity using the initial potential of −0.8 V.27, 28 The ECL maximum emission wavelength is about 440 nm (Figure 2), which is consistent with that reported in literatures.1, 2
the dependence of ECL intensities on pH is attributed to the dependence of these reactions on pH and the deprotonation of luminol.

**Fig. 1** ECL behaviors of luminol (A) and cyclic voltammograms (B) by scanning in different potential ranges and directions: (a) from -0.8 V to 1.0 V to -0.8 V; (b) from 0 V to 1.0 V to 0 V; (c) from 0 V to -0.8 V to 0 V; (d) from -0.8 V to 1.0 V to -0.8 V in deaerated solutions. Conditions: c(luminol): 1 \times 10^{-5} M; Phosphate buffer: 0.2 M, pH 10.0; Photomultiplier tube voltage: 900 V.

**Fig. 2** ECL spectrum of luminol on glassy carbon electrode by scanning potential from -0.8 V to 1.0 V. Scan rate: 0.1 V/s; c(luminol): 1 \times 10^{-5} M; Phosphate buffer: 0.2 M, pH 10.0; Photomultiplier tube voltage: 1100 V.

Figure 3 shows the effect of pH on ECL intensities. The ECL intensities increase with increasing pH value, and level off at pH 10.0. ECL reactions involve in the electrochemical reduction of oxygen, the electrochemical oxidation of luminol and electrogenerated ROS, as well as the reactions between luminol and ROS. These reactions depend significantly on pH. Therefore, the dependence of ECL intensities on pH is attributed to the dependence of these reactions on pH and the deprotonation of luminol.

**Fig. 3** Effect of pH on ECL intensities at glassy carbon electrode by scanning potential from -0.8 V to 1.0 V. Scan rate: 0.1 V/s; c(luminol): 1 \times 10^{-5} M; Phosphate buffer: 0.2 M; Photomultiplier tube voltage: 900 V.

**Fig. 4** Effect of scan rate on ECL intensities on glassy carbon electrode by scanning potential from -0.8 V to 1.0 V. c(luminol): 1 \times 10^{-5} M; Phosphate buffer: 0.2 M, pH 10.0; Photomultiplier tube voltage: 900 V.

Figure 4 shows the effects of scan rates on ECL. The ECL intensities increase sharply and reach the highest value at a scan rate of 0.10 V/s, and then decrease when the scan rates are over 0.10 V/s. At scan rates increase, the concentrations of intermediates generated from the electrochemical oxidation of luminol increases, leading to the increase in ECL intensities. However, as the scan rates further increase, less ROS is generated, resulting in the decrease in ECL intensities at high scan rates.

**Fig. 5** shows the influence of initial potentials on luminol ECL. The ECL intensities increase remarkably as the initial potentials decrease from 0 to -0.8 V, and then decrease as the initial potentials decrease further. The ECL intensity using an initial potential of -0.8 V is about 470-fold of that using an initial potential of 0.0 V. The increases in ECL intensities as initial potentials shift from 0 to -0.8 V are attributed to the generation of more ROS. The decreases in ECL intensities as initial potentials shift from -0.8 V to -1.2 V are attributed to the generation of less ROS since more oxygen molecules were

<table>
<thead>
<tr>
<th>Scan Rate / V/s</th>
<th>ECL Intensity / a.u.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1000</td>
</tr>
<tr>
<td>0.08</td>
<td>5000</td>
</tr>
<tr>
<td>0.12</td>
<td>8000</td>
</tr>
<tr>
<td>0.16</td>
<td>1000</td>
</tr>
</tbody>
</table>

Figure 5 shows the influence of initial potentials on luminol ECL. The ECL intensities increase remarkably as the initial potentials decrease from 0 to -0.8 V, and then decrease as the initial potentials decrease further. The ECL intensity using an initial potential of -0.8 V is about 470-fold of that using an initial potential of 0.0 V. The increases in ECL intensities as initial potentials shift from 0 to -0.8 V are attributed to the generation of more ROS. The decreases in ECL intensities as initial potentials shift from -0.8 V to -1.2 V are attributed to the generation of less ROS since more oxygen molecules were
Since luminol and its derivatives are popular labels for bioassays, such as immunoassays and DNA probe assays, we thus use the proposed method to detect luminol. ECL intensities increase linearly with increasing luminol concentrations from 1 nM to 2.5 μM with a correlation coefficient (r) of 0.9976. The regression equation is \( I = 29.00 + 1180.50 \times c(\mu M) \) and the limit of detection (LOD) is \( 3 \times 10^{-10}\text{M} \). Compared with other ECL methods (Table 1), the proposed method in this study is more sensitive than other methods for the detection of luminol. Moreover, this method does not require the modification of electrode, significantly simplifying detection.

**Table 1.** Comparison of different glassy carbon electrodes for the ECL detection of luminol.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Linear ranges ((\text{nM}))</th>
<th>LOD ((\text{nM}))</th>
<th>( \text{H}_2\text{O}_2 )</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT-Nafion-modified GCE</td>
<td>0.05–100μM</td>
<td>9.2</td>
<td></td>
<td>[24]</td>
</tr>
<tr>
<td>u-FePc nanostructure fabricated GCE</td>
<td>0.01–10μM</td>
<td>5</td>
<td>√</td>
<td>[29]</td>
</tr>
<tr>
<td>TPP/MWNT modified GCE</td>
<td>0.05–8 μM</td>
<td>10</td>
<td></td>
<td>[30]</td>
</tr>
<tr>
<td>Nickel phthalocyanine modified GCE</td>
<td>0.1–8μM</td>
<td>60</td>
<td>√</td>
<td>[31]</td>
</tr>
<tr>
<td>Bare GCE</td>
<td>1nM–2.5μM</td>
<td>0.3</td>
<td></td>
<td>Present work</td>
</tr>
</tbody>
</table>

**Conclusion**

In conclusion, we have developed a simple method to dramatically increase luminol ECL by coupling electrochemical reduction and electrochemical oxidation using simple linear sweep voltammetry. The remarkable increases in ECL intensities significantly improve the sensitivity for the detection of luminol. This detection scheme is appealing for bioassays using luminol labels. The generation of intense luminol ECL by this method facilitates ECL detection using less sensitive, cheaper, and portable ECL detectors. Moreover, this detection strategy is very promising for ECL of other luminophores, such as lucigenin, peroxoxalates, and nanoluminophores.

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