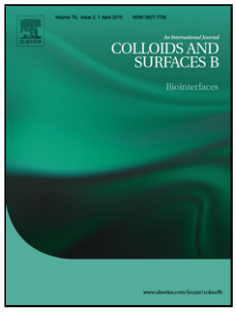

Title: A widened emission window of the peroxydisulfate-oxygen system for the detection of *L*-alanine

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A widened emission window of the peroxydisulfate-oxygen system for the detection of *L*-alanine

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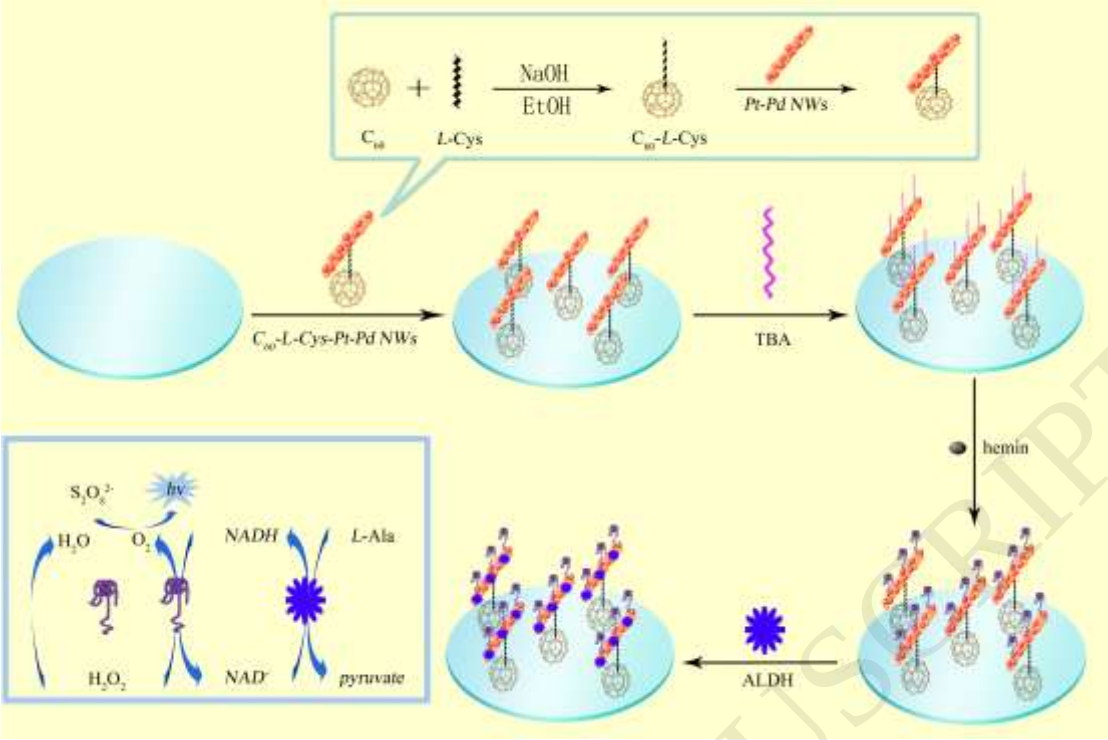
Graphical abstract



Graphical abstract

Illustration of the designed biosensor based on signal amplification strategy for detecting *L*-Ala

Highlights

- ● ECL stereoselective discrimination and detection of *L*-Ala is achieved.
- ● The adopted signal amplification complex could greatly amply the ECL response.
- ● The widened emission window may expand the scope of target analytes.
- ● The applied potential may open a new view to apply ECL technology in other fields.

Abstract:

Peroxydisulfate-oxygen ($S_2O_8^{2-}$-O_2) system has become one of the most used systems in electrogenerated chemiluminscence (ECL) field. Due to $S_2O_8^{2-}$ can be used as Fenton Reagent, this work designed an ECL biosensor based on the $S_2O_8^{2-}$-O_2 system for the detection of *L*-alanine in a widened emission window and using hemin/G-quadruplex and platinum and palladium nanowires (Pt-Pd NWs) to in situ generate O_2 to amplify the ECL intensity. The proposed ECL sensor showed an excellent analytical property for the detection of *L*-alanine in a linear range of 5.0×10^{-3} M to 1.0×10^{-8} M with the detection limit of 3.3×10^{-9} M (S/N=3). This work with high selectivity, stability and reproducibility may open a new door to apply $S_2O_8^{2-}$ in ECL field.

***Keywords*:** electrogenerated chemiluminscence; *L*-alanine; signal amplification complex; peroxydisulfate-oxygen

1. Introduction

Chirality is a fundamental chemical property in the living system, and it is very important for deep understanding the interactions between biological molecules. Amino acids play central roles in living creatures owing to it is the building block of many biological molecules. For instance, *L*-alanine (*L*-Ala) is a crucial factor in amino acid metabolism and it can be used as a nutrition in pharmaceutical field or an

additive in food additive industry [1]. Recently, the method of using microorganism to produce *L*-Ala has been reported, but the excessive *L*-Ala is toxic in the period of microorganism growth [2, 3]. To avoid a toxic-level accumulation of intracellular *L*-Ala, the real-time analysis of *L*-Ala is necessary.

Many methods have been used to detect *L*-Ala, for example, electrochemistry (EC) technology, UV/Vis spectrometry (UV/Vis), and electrogenerated chemiluminscence (ECL) [4-7]. Compared with other methods, ECL as a powerful detection method has gradually aroused the researchers' concern because of its high sensitivity and signal-to-noise (S/N) ratio. In various organic and inorganic ECL systems, peroxydisulfate-oxygen ($S_2O_8^{2-}$-O_2) system has been popularly applied due to the advantages of simplicity, sensitivity and cheapness [8]. Nevertheless, the coreactant of $S_2O_8^{2-}$-O_2 system, dissolved O_2, is suffered from difficulty of low concentration in the detection solution. Thus, a strategy of in situ generating O_2 is a fascinating method to overcome the above short-coming [9-11].

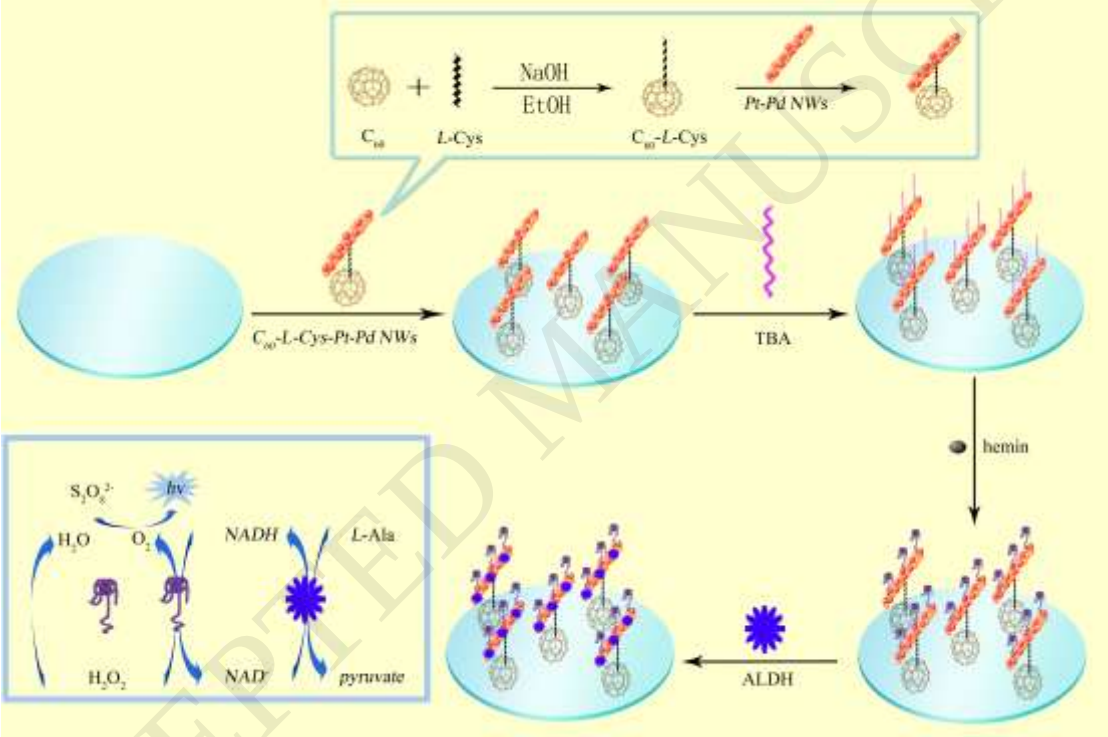
Artificial mimetic enzymes have seen great interest from the research community as catalysts for amplifying sensing events, especially the hemin/G-quadruplex formed by guanine-rich nucleic acid sequences and hemin [12, 13]. It can act not only as a peroxidase, but also a β-nicotinamide adenine dinucleotide hydrogen (NADH) oxidase [14, 15]. Based on these characters, hemin/G-quadruplex has often been applied to enhance the emission of ECL system [16-18].

In addition, nanomaterials have significant effect in the construction of biosensors for signal amplifacation. For instance, carbon nanomaterials with a high surface area

and superior conductivity can guarantee the immobilization of other composition. Fullerene (C_{60}), as a member of the carbon family, is a hollow carbon sphere rich in conjugated π electrons. With a high symmetrical structure and unique chemical/physical properties, C_{60} can react with amines and decorate with metal materials [19, 20]. After C_{60} has been decorated by *L*-cysteine (*L*-Cys) to form the fullerene derivative (C_{60}-*L*-Cys), the solubility can be greatly improved, and it can be used as an ECL signal amplified reagent for $S_2O_8^{2-}$-O_2 system [21-24]. Another kind of material which adopted frequently is noble-metal nanomaterials. Such as bimetallic nanowires with one-dimensional morphologies can improve some inherent catalytic problems which exist in state-of-the-art nanopaticulate catalysts [25]. Here, a simple and one-pot solution synthesis of one dimensional nanowires of platinum and palladium nanowires (Pt-Pd NWs) has been used as a signal amplification materials owing to the excellent electrocatalytic performance, large surface area and favorable biocompatibility [26].

In this work, a novel ECL biosensor has been developed by *L*-alanine dehydrogenase (ALDH) and hemin/G-quadruplex that simultaneously acted as NADH oxidase as well as peroxidase to in situ generate coreactant. Additionally, C_{60}-*L*-Cys and Pt-Pd NWs were used to prepare C_{60}-*L*-Cys-Pt-Pd NWs nanocarrier, so as to apply it to load up hemin/G-quadruplex and ALDH to fabricate a sensitive ECL sensor for the detection of *L*-Ala. Based on $S_2O_8^{2-}$-O_2 system, in which potential range from -2.0 to 0.0 V always be taken as an effective emission window in the conventional experiments, the highest ECL intensity of this work have been obtained

in a lower oxidation potential (-2.5 to -3.1 V). This lower potential range only be used in water treatment, in which peroxydisulfate has been used as a Fenton reagent to produce the strong oxidizing species (sulfate radicals, $SO_4^{\bullet-}$) [27-37]. That is to say, a widened emission window of the $S_2O_8^{2-}$-O_2 system has been applied in the detection of *L*-alanine, it may open a new avenue to apply ECL technology. The process of stepwise assembly of the biosensor and the detection principle were shown in **Scheme 1**.



Scheme 1. The fabrication process and the reaction mechanism of the biosensor

2. Results and discussion

2.1 Enantioselective interaction of Ala enantiomers

The ECL responses of the chiral interfaces to Ala enantiomers have been investigated in 0.1 M PBS (pH 7.4) containing peroxydisulfate and 5.0×10^{-3} M NAD^+ with the potential sweeping from -3.0 to 0.0 V. As shown in **Fig. 1**, the changed

ECL intensities were obtained. Only a slightly higher intensity was obtained in *D*-Ala solution (curve b) than that in reagent blank (0.1 M PBS, pH 7.4, containing peroxydisulfate and 5.0×10^{-3} M NAD^+) (curve a), while a very high ECL intensity was obtained in the presence of *L*-Ala (curve c). This is due to the stereospecific oxidative deamination of ALDH to *L*-Ala.

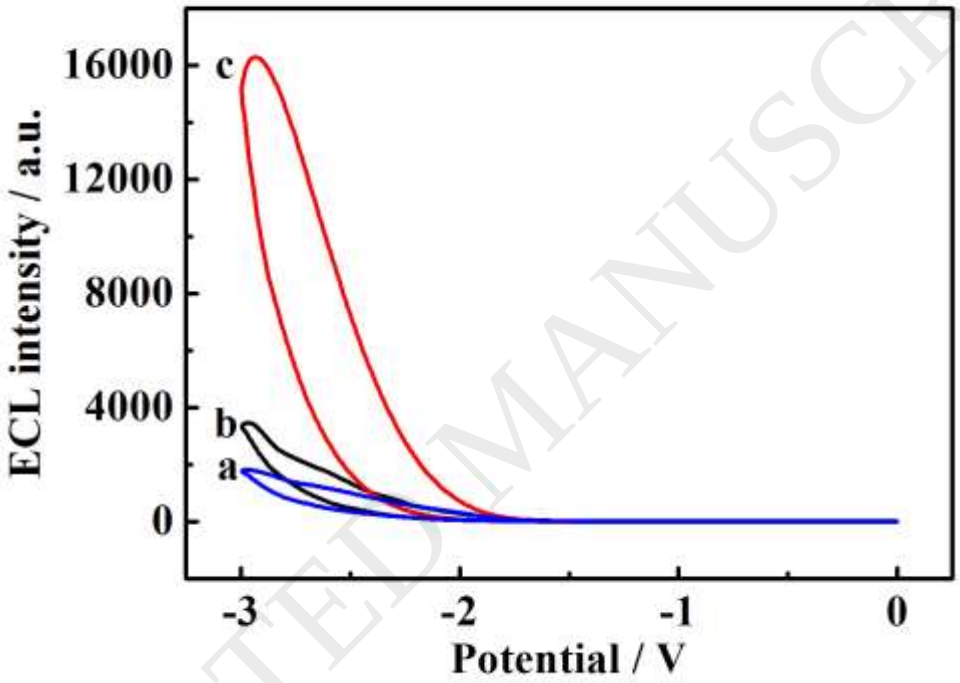


Fig. 1. ECL intensities of ALDH/hemin/TBA/C_{60}-*L*-Cys-Pt-Pd NWs/GCE in (a) blank, 5.0×10^{-3} M of (b) *D*-Ala and (c) *L*-Ala

2.2 Possible mechanism for the amplification of the ECL signal

The possible light emission mechanism of the ECL process was described below: *L*-Ala was stereospecificly oxidized by ALDH to generate NADH, Subsequently, NADH was rapidly oxidized by hemin/G-quadruplex and accompained to generate

H_2O_2. And H_2O_2 was catalyzed by hemin/G-quadruplex to in situ generate $HOO^{\bullet}$, which could act as a coreactant of $S_2O_8^{2-}$ in ECL emission system [20, 38-41].

$$L\text{-Ala} + NAD^+ \xrightarrow{ALDH} \text{pyruvate} + NH_3 + NADH$$

$$NADH + O_2 + H^+ \xrightarrow{\text{hemin/G-quadruplex}} NAD^+ + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{hemin/G-quadruplex}} HOO^{\bullet}$$

$$S_2O_8^{2-} + e \rightarrow SO_4^{\bullet-} + SO_4^{2-}$$

$$SO_4^{\bullet-} + H_2O \rightarrow HO^{\bullet} + HSO_4^-$$

$$HO^{\bullet} + H_2O \rightarrow HOO^{\bullet} + H_2$$

$$O_2 + H_2O + e \rightarrow HOO^{\bullet} + HO^-$$

$$SO_4^{\bullet-} + HOO^{\bullet} \rightarrow HSO_4^- + {}^1(O_2)_2{}^*$$

$${}^1(O_2)_2{}^* \rightarrow 2{}^3O_2 + h\nu$$

Along with the enhanced effection of C_{60}-*L*-Cys and Pt-Pd NWs, more and more H_2O_2 could be catalysed to produce a great deal of ${}^1(O_2)_2{}^*$:

$$H_2O_2 \xrightarrow{\text{Pt-Pd NWs}} HOO^{\bullet}$$

$$C_{60}\text{-}L\text{-Cys} - e \rightarrow C_{60}\text{-}L\text{-Cys}^{\bullet+}$$

$$C_{60}\text{-}L\text{-Cys}^{\bullet+} \rightarrow C_{60}\text{-}L\text{-Cys}^{\bullet} + H^+$$

$$C_{60}\text{-}L\text{-Cys}^{\bullet} + HOO^{\bullet} \rightarrow {}^1(O_2)_2{}^*$$

It is worth to notice that under the potential range of -2.0 to 0.0 V, which always be used as an effective emission window of $S_2O_8^{2-}$-O_2 system in the usual experiments, no good response can be acquired, the highest intensity have been obtained nearby -3.0 V. The ECL emission potential is negatively shifted to -3.0 V. In this lower oxidation potential, more $SO_4^{\bullet-}$ (the intermediate product) would be provided by

$S_2O_8^{2-}$-O_2 system [27-31], and more $HO^{\bullet}$, $HOO^{\bullet}$, and $^1(O_2)_2^*$ could be gotten. So, the light emission would be enhanced greatly. That is to say, the emission window of $S_2O_8^{2-}$-O_2 system has been widened in this work.

To further investigate this phenomenon, role of each component has been discussed. **Fig. 2A** shows the ECL behavior of different electrodes which modified by different materials in 0.1 M PBS containing 0.1 M $S_2O_8^{2-}$, 5.0 × 10^{-3} M NAD^+ and *L*-Ala solution. All signals were observed nearby -3.0 V. Bare GCE got a very low ECL intensity (curve a). While the modified electrode showed an excellent ECL response (curve e). After ALDH has been removed, the ECL signal decreased dramatically (curve d). This may because *L*-Ala cannot react directly with the rest materials so that the followed reaction stopped. When hemin/G-quadruplex has not been incubated, the prepared electrode gained much lower signal (curve c) as the produced NADH could not be catalysed without hemin/G-quadruplex. When there was only C_{60}-*L*-Cys-Pt-Pd NWs complex, the minimal signal has been obtained (curve b). Hinting each component owned perfect catalytic ability in the emission system.

Simultaneously, corresponding CV responses have also been discussed. From **Fig. 2B**, when $E° \leq -1.5$ V, in the region where reduction of peroxydisulfate may take place [42], a reduction peak was observed at bare GCE (curve a). When there was only C_{60}-*L*-Cys-Pt-Pd NWs complex, the current was increased because of the conductivity of it (curve b). The current was decreased when ALDH or hemin/G-quadruplex presented (curve c and curve d). Due to hemin/G-quadruplex can cause the reduction of the dissolved O_2, which served as the supplying resources of light-emitting species

$^1(O_2)^*_2$[40, 41]. The CV response of hemin/G-quadruplex/C_{60}-*L*-Cys-Pt-Pd NWs/GCE was higher than ALDH/C_{60}-*L*-Cys-Pt-Pd NWs/GCE.

These facts mentioned above proved that each component of the materials did their jobs well in the light emission process, and greatly amplied the response signals of $S_2O_8^{2-}$-O_2 system.

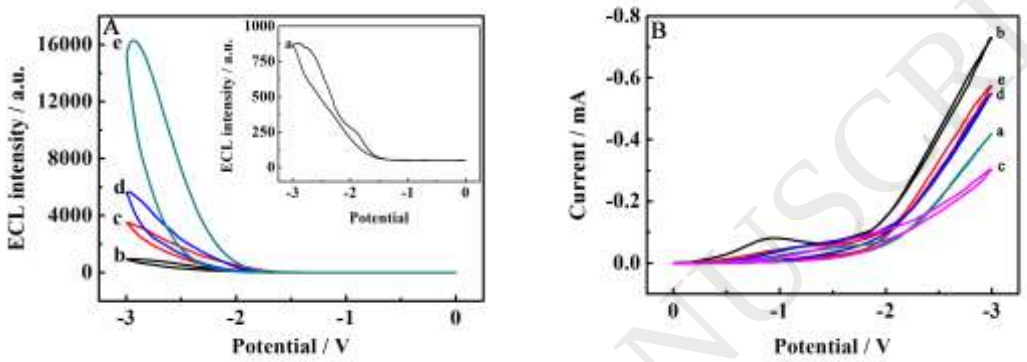


Fig. 2. ECL (A) and CV responses (B) of different electrodes: (a) bare GCE, (b) C_{60}-*L*-Cys-Pt-Pd NWs/GCE, (c) ALDH/C_{60}-*L*-Cys-Pt-Pd NWs/GCE, (d) hemin/TBA/C_{60}-*L*-Cys-Pt-Pd NWs/GCE and (e) ALDH/hemin/TBA/C_{60}-*L*-Cys-Pt-Pd NWs/GCE

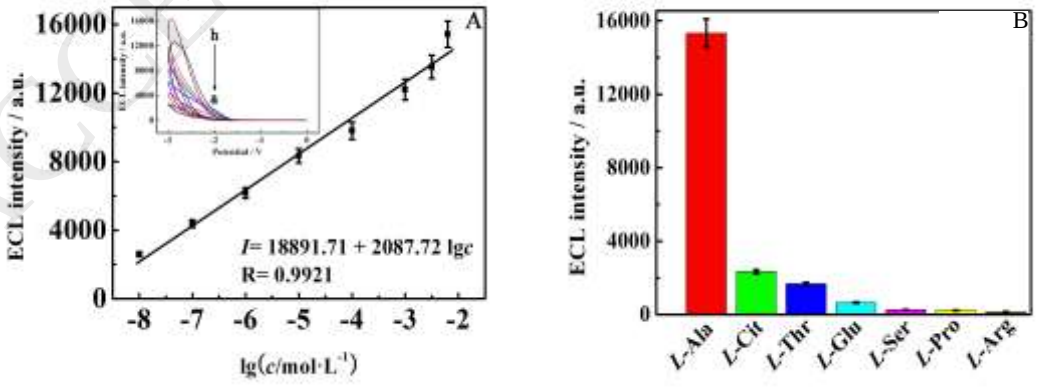
It was reported that crystal shape may affect the the emission potential [19], thus Pt-Pd NWs were replaced by Pt-Pd nanoparticles (Pt-Pd NPs) and it was observed that the emission peak potential moved slightly in the oxidation range of $SO_4^{\bullet-}$ (**Fig. S3**). In summary, the oxidation potential of the intermediate $SO_4^{\bullet-}$ and the shape of nanomaterials may attribute to the widened of the emission window of $S_2O_8^{2-}$-O_2 system.

2.3 Analytical performance of the modified electrodes

As shown in **Fig. 3A**, the analytical performance of the modified electrodes were

investigated by detecting the target *L*-Ala at various concerntrations. A promised linear regression equation of ECL response signals on -3.0 V were obtained. The ECL intensities were increased with the increasing *L*-Ala concentration from 1.0×10^{-8} to 5.0×10^{-3} M. The linear regression equation was I=18891.71 + 2087.72 lg$c$ with the correlation coefficient of R^2=0.9921 and a detection limit of 3.3×10^{-9} M (S/N=3). The experimental results showed some target analytes which cannot obtained a satisfied linear relationship may be detected on a wider emission window.

The selectivity of the designed electrodes were investigated by testing the ECL signals in a series of *L*-amino acids with a concentration of 5.0×10^{-3} M. As shown in **Fig. 3B**, the ECL intensity of *L*-Ala is the largest. Obviously, the proposed electrode possessed a satisfactory selectivity. The stability of the biosensor ECL intensity in the presence of 5.0×10^{-3} M *L*-Ala is showed in **Fig. 3C**, The reproducibility of the modified electrodes were also studied. Consequently, similar ECL respons was acquired and the relative standard deviation (RSD) is less than 4.0 %, indicating the acceptable reproducibility of the modified electrode for detecting *L*-Ala.



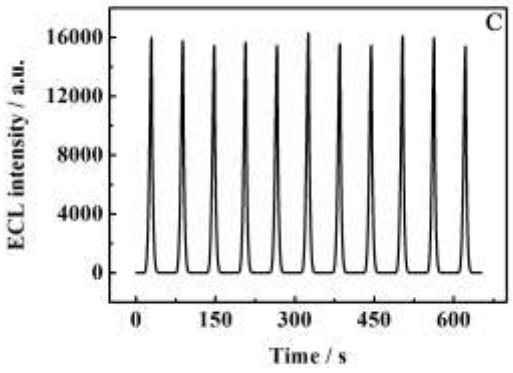


Fig. 3. ECL responses of the proposed biosensor to (A) different concentrations of *L*-Ala in 0.1 M PBS (pH 7.4) containing 0.1 M peroxydisulfate and 5.0 $\times 10^{-3}$ M NAD^+: (a) 1.0 $\times 10^{-8}$, (b) 1.0 $\times 10^{-7}$, (c) 1.0 $\times 10^{-6}$, (d) 1.0 $\times 10^{-5}$, (e) 1.0 $\times 10^{-4}$, (f) 1.0 $\times 10^{-3}$, (g) 3.0 $\times 10^{-3}$ and (h) 5.0 $\times 10^{-3}$ M; (B) different *L*-amino acids with the concentration of 5.0 $\times 10^{-3}$ M. (C) ECL stability of the modified electrode to the 5.0 $\times 10^{-3}$ M *L*-Ala.

3. Conclusion

In summary, this work designed a widen emission window of $S_2O_8^{2-}$-O_2 system for detecting *L*-Ala. With a strategy of signal amplification, the fixed abundant O_2 was in situ generated and the highest ECL response was obtained on nearby -3.0 V, in which may expand the scope of target analytes. According to the results, the designed biosensor possessed a good performance with wide linear range, outstanding sensitivity, acceptable reproducibility and stability, and it will pave the way for the development of ECL biosensors to detect *L*-Ala in pharmaceutical field and food additive industry.

Acknowledgments

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