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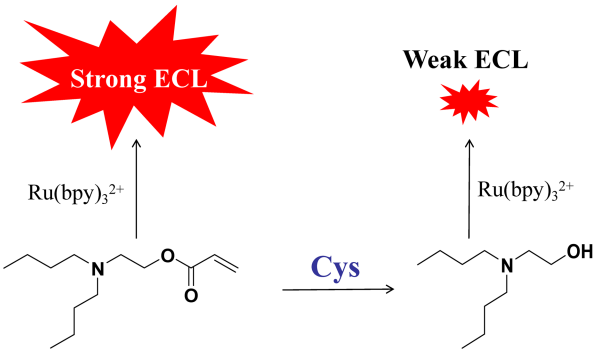

2-(Dibutylamino)ethyl acrylate as a highly efficient co-reactant of $Ru(bpy)_3^{2+}$ electrochemiluminescence for selective detection of cysteine

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Abstract

A highly efficient co-reactant of $Ru(bpy)_3^{2+}$ electrochemiluminescence (ECL), 2-(dibutylamino)ethyl acrylate(DBAEA), was successfully synthesized by a straightforward one-step reaction between dibutylaminoethanol and acrylyl chloride. On account of the interaction between the olefin group of DBAEA and Pt electrode,

the ECL intensity utilizing DBAEA as the co-reactant was increased dramatically, nearly as 10 times and 17 times as conventional co-reactants dibutyaminoethanol and tripropylamine. Since cysteine can remove the acrylate group of DBAEA specifically through the conjugated addition/cyclization reaction, the ECL intensity of $Ru(bpy)_3^{2+}$/DBAEA was declined selectively. Therefore, cysteine was detected through this novel ECL system with high selectivity towards other amino acids. The linear range was between 10 μM to 200 μM with a limit of detection of 1.15 μM. The cysteine in Dulbecco's Modified Eagle Medium (DMEM) was detected and satisfying recovery was obtained.

Key words: Electrochemiluminescence; 2-(Dibutylamino)ethyl acrylate; Cysteine; Co-reactant; Olefin group

1. Introduction

Electrochemiluminescence (ECL) is an efficient analytical method with the advantages of low background, high sensitivity, simplicity, rapidity, easy miniaturization and integration [1]. $Ru(bpy)_3^{2+}$ has good stability, solubility and ECL efficiency in aqueous solutions. In addition, $Ru(bpy)_3^{2+}$ ECL allows the detection of various co-reactants such as proteins, amino acids, DNA and pharmaceuticals, making it one of the mostly used luminophores [2-5].

Cysteine (Cys) plays an important role in metabolism, detoxification and protein synthesis [6-8]. The amount of Cys in the body is related to various diseases, such as cardiovascular disease, Alzheimer’s disease and liver damage [9, 10]. Hence, it is necessary to develop efficient methods to detect Cys quantitatively. Currently, several ECL-based methods for the detection of Cys have been reported [11-13]. However,

those methods depend on the interactions, such as hydrogen bond, Van der Waal's force and electrostatic attraction between Cys and luminophores or co-reactants, which limits the selectivity.

Herein, a new ECL co-reactant, 2-(dibutylamino)ethyl acrylate(DBAEA), was synthesized through the reaction between dibutylaminoethanol (DBAE) and acrylyl chloride. DBAEA can dramatically increase the ECL intensity of $Ru(bpy)_3^{2+}$ on Pt electrode compared with tripropylamine (TPA) and DBAE. This increase was attributed to the strong interaction of olefinic group with Pt electrode, which made the electro-oxidation of DBAEA easier. Cys can remove the acrylate group of DBAEA through a Michael addition/cyclization reaction between thiol and acrylate group [14-16], leading to the decrease of ECL intensity. As a consequence, Cys can be quantitatively detected by the decrease of ECL intensity (Scheme 1). It is worth mentioning that this simple detection system exhibited outstanding selectivity towards amino acids and possessed high sensitivity.

2. Experimental

2.1 Chemicals and Apparatus

Both dibutylaminoethanol and tris(2,2'-bipyridyl) ruthenium(II) chloride were purchased from Sigma-Aldrich. Tripropylamine and acryloyl chloride were obtained from Aladdin (Shanghai, China). Cysteine was provided by Shanghai Yuanju biological company (China). Other amino acids, such as alanine, glutamic, aspartic, were bought from Beijing Dingsheng biological company (China). $Na_2HPO_4 \cdot 12H_2O$ and NaH_2PO_4 were obtained from Beijing Chemical Reagent company(China). Dichloromethane and dimethyl sulfoxide (DMSO) were provided by Tianjin Fuyu Fine Chemical Company (China). Dulbecco's Modified Eagle Medium (DMEM) was

obtained from Beijing Wobisen Technology Company. All the reagents and chemicals were of analytical grade without any further purification. The deionized water and 0.1 M phosphate buffer solution (PBS) were utilized throughout the whole experiment. The structure of DBAEA was confirmed by high-resolution mass spectrometry (MS) and proton nuclear magnetic resonance (^{1}HNMR). The ^{1}HNMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer. High-resolution mass spectrum was determined on an Bruker Autoflex Ⅲ MALDI-TOF mass spectrometer.

2.2 Synthesis of DBAEA

DBAEA was synthesized according to the reported method (Scheme 2) [17]. Briefly, 0.1 mol DBAE, 0.1 mol triethylamine and 0.001 mol hydroquinone were dissolved in 100 mL tetrahydrofunan (THF) into a dry flask. Then 0.1 mol acryloyl chloride was added dropwise under room temperature. After the reflux for 2 hours, triethylamine salt was removed by filtration and the solvent was evaporated. The obtained reaction product was distilled in vacuum between 83 ℃ to 87 ℃. The final product DBAEA was presented as yellow liquid and dissolved in DMSO for further adoption.

2.3 Procedure of ECL detection

In a typical ECL experiment, 50 μL $Ru(bpy)_3^{2+}$, 50 μL DBAEA of desired concentration and 0.1 M PBS were pipetted into the ECL cell to make the final solution 500 μL. The cyclic voltammetry (CV) was performed on a CHI 800B potentiostat with the potential ranging from 0 V to 1.5 V and a scan rate of 100 mV/s unless special illustration. The ECL intensity was detected on an ultra-weak luminescence analyzer. The PMT was biased at 1000 V.

2.4 The detection of Cys

In each experiment of Cys detection, 50 μL DBAEA (50 mM) and 50 μL Cys were mixed with 100 μL 0.1 M pH 7.0 PBS and the mixture was kept for a period to react completely. Subsequently, the solution was mixed with 50 μL $Ru(bpy)_3^{2+}$ (10 μM) and 250 μL 0.1 M pH 7.0 PBS into the ECL cell.

3. Results and discussion

DBAEA was prepared by Michael addition reaction between DBAE and acrylyl chloride (Scheme 2). The structure of DBAEA was confirmed by ^1H NMR (Figure S1) and MS (Figure S2).

3.1 The ECL and CV performances of TPA, DBAE and DBAEA as co-reactants on different electrodes

The ECL intensities of $Ru(bpy)_3^{2+}$ with different co-reactants were compared on Pt electrode, glassy carbon electrode (GCE) and Au electrode (Figure 1). The $Ru(bpy)_3^{2+}$/DBAEA ECL intensity is nearly 17 times and 10 times of $Ru(bpy)_3^{2+}$/TPA and $Ru(bpy)_3^{2+}$/DBAE on Pt electrode and 7 times and 4 times on Au electrode. However, the difference between co-reactants is not obvious on GCE. The CV curves of three ECL systems on different electrodes were also studied (Figure 2). The oxidation current intensity of DBAEA is the highest, and next come DBAE and TPA separately on three electrodes. Olefin is commonly used to modify metal electrodes especially Pt electrode through chemical adsorption [18-20]. The interaction between the olefinic group of DBAEA and metal electrodes may affect the amount of electric

charge passed through the solution. Moreover, the olefinic group of DBAEA may enable more efficient generation of the excited $Ru(bpy)_3^{2+}$* during electron transfer annihilation between electrogenerated $Ru(bpy)_3^{3+}$ and $DBAEA^{\bullet}$ produced by proton abstraction from $DBAEA^{\bullet+}$ (Scheme 3). Therefore, DBAEA shows much stronger ECL than DBAE and TPA at Pt and Au electrodes.

3.2 The ECL intensity of $Ru(bpy)_3^{2+}$ with different concentrations of TPA, DBAE and DBAEA as co-reactants

The $Ru(bpy)_3^{2+}$ ECL intensities with TPA, DBAE and DBAEA as co-reactants of different concentrations on Pt electrode were compared in Figure 3. TPA has the weakest increasing effect and the slowest increasing speed. The ECL intensity of $Ru(bpy)_3^{2+}$ increases with DBAE concentrations and becomes the strongest when the concentrations exceed 20 mM, which is consistent with the previous work of our group [21]. However, the ECL intensity of $Ru(bpy)_3^{2+}$/DBAEA is much more higher and reaches 17 times and 10 times as that of $Ru(bpy)_3^{2+}$/TPA and $Ru(bpy)_3^{2+}$/DBAE at a co-reactant concentration of 5 mM. The slight decrease of ECL intensity of $Ru(bpy)_3^{2+}$/DBAEA at DBAEA concentrations higher than 5 mM may be ascribed to side reactions, such as radical annihilation by olefinic group of DBAEA. Therefore, 5 mM of DBAEA was adopted as optimized concentration in the following experiments.

3.3 The ECL mechanism of $Ru(bpy)_3^{2+}$/DBAEA

The ECL mechanism of $Ru(bpy)_3^{2+}$/DBAEA was inferred as demonstrated in Scheme 3 according to some common tertiaryamine co-reactants [22]. Firstly,

$Ru(bpy)_3^{2+}$ and DBAEA are electro-oxidized as $Ru(bpy)_3^{3+}$ and $DBAEA^{\bullet}$, respectively. Then $DBAEA^{\bullet}$ reacts with $Ru(bpy)_3^{3+}$, generating the excited state of $Ru(bpy)_3^{2+*}$. Finally $Ru(bpy)_3^{2+*}$ turns back to the ground state accompanied by light emitting. It has been proved that the direct oxidation of co-reactants is dominant at low concentration of $Ru(bpy)_3^{2+}$ and the electro-oxidation of co-reactant by $Ru(bpy)_3^{2+}$ is dominant at high concentration of $Ru(bpy)_3^{2+}$ [23-26]. Since the concentration of $Ru(bpy)_3^{2+}$ (1 μM) in this work is much lower than that of DBAEA (5 mM), the direct electrooxidation of DBAEA is dominant. As shown in Figure S3, the anodic current varies approximately linearly with the square root of the scan rate ($v^{1/2}$), demonstrating that diffusion process plays important role in ECL in the presence of the relatively high concentration of DBAEA.

3.4 The ECL property of $Ru(bpy)_3^{2+}$/DBAEA system with and without Cys

The influence of Cys on the ECL intensity of $Ru(bpy)_3^{2+}$/DBAEA system was investigated. As demonstrated in Figure 4A, both individual $Ru(bpy)_3^{2+}$ and DBAEA has weak ECL intensity and the ECL intensity of $Ru(bpy)_3^{2+}$/DBAEA is much higher. After the reaction between Cys and DBAEA, the ECL intensity is declined by nearly 7 times. The current intensity of different components (Figure 4B) is consistent with the results of ECL system. That is, the current intensity of $Ru(bpy)_3^{2+}$/DBAEA system is higher than that of $Ru(bpy)_3^{2+}$ and DBAEA, and the addition of Cys can decrease the current. It is obvious that the characteristic oxidation peak of Cys at around 0.6 V cannot be seen in the reacting mixture. This phenomenon suggests that the decrease of ECL is caused by the reaction between Cys and DBAEA.

3.5 The mechanism of the reaction between Cys and DBAEA

It has been reported that Cys can remove the acrylate group of compound specifically through Michael addition/cyclization reaction [14-16]. The mechanism of the reaction between DBAEA and Cys is displayed as two steps according to the previous report (Scheme 4). The first step is Michael addition. The nucleophilic reagent thiol attacks and links to the unsaturated β-carbon atom of carbonyl. The electron moves along carbon chain and generates unstable oxygen anion. Next it turns into carbon anion and combines with a proton to become saturated carbonyl carbon. The second step is cyclization. Briefly, amine serves as a nucleophilic reagent to attack the carbonyl carbon to generate a heptatomic ring. Finally, the ring leaves and the acrylate is removed successfully.

To prove the mechanism, we mixed DBAEA and Cys for over 10 minutes and checked the ^{1}H NMR spectrum (Figure S4). As we can see, the characteristic olefin peaks disappeared compared with the ^{1}H NMR spectrum of DBAEA, indicating that the olefin group was removed by Cys successfully.

3.6 The optimization of the conditions for the detection of Cys

To obtain higher sensitivity for Cys detection, the influence of several experimental conditions were studied to find out the optimized ones (Figure S6). For the balance of wide linear detection range and low LOD, each detection was performed under the concentration of 5 mM DBAEA.

The optimized concentration of $Ru(bpy)_3^{2+}$ was studied by measuring the ECL intensity before and after the reaction of DBAEA and Cys under different concentrations of $Ru(bpy)_3^{2+}$ (0.5 μM, 1 μM, 2 μM, 3 μM, 5 μM) (Figure S5A). The ECL intensity ratio of $Ru(bpy)_3^{2+}$/DBAEA in the presence and absence of Cys is the biggest when the concentration of $Ru(bpy)_3^{2+}$ is 1 μM (Figure S5B). Therefore, the

concentration of $Ru(bpy)_3^{2+}$ was set as 1 μM in subsequent experiments.

The influence of pH on the detection of Cys was also investigated in the pH range from 6.5 to 10.0 (Figure S5C). It can be observed that the ECL intensity of $Ru(bpy)_3^{2+}$/DBAEA is the strongest when pH is 7.5 with or without Cys. However, the ECL intensity ratio of $Ru(bpy)_3^{2+}$/DBAEA in the presence and absence of 5 mM Cys is the biggest when pH is 7.0 (Figure S5D). It is because the thiol of Cys can be easily oxidized in alkaline condition, restricting the reaction of Cys and DBAEA, and Michael addition is difficult to proceed in acid condition. To get higher sensitivity, a pH of 7.0 was utilized in the following experiments.

The reaction time is another crucial condition for Cys detection. As illustrated in Figure S5E, the ECL intensity increases with time and levels off after 10 minutes, indicating the reaction proceeds completely within 10 minutes. Hence, each ECL detection was performed after mixing Cys and DBAEA for 10 minutes.

3.7 The detection of Cys

Cys was detected under optimized conditions by studying the ECL intensity decrease caused by Cys. As demonstrated in Figure 5A, the decreasing value (I_1-I_2) increases with the concentrations of Cys. There is a linear relationship between the logarithm of (I_1-I_2) and the logarithm of Cys concentrations from 10 μM to 200 μM (Figure 5B). The linear equation is $\log (I_1-I_2) = 3.871 + 0.816 \log C$ ($R^2 = 0.994$), with a limit of detection (LOD) of 1.15 μM. The comparison of our method with other methods for Cys detection (Table 1) suggests that this method is sensitive.

3.8 The selectivity of $Ru(bpy)_3^{2+}$/DBAEA ECL system

In order to prove the excellent selectivity of this method towards Cys, we studied

the influence of other amino acids on the ECL intensity of $Ru(bpy)_3^{2+}$/DBAEA following the same procedure as Cys. As shown in Figure 6, only Cys can lead to obvious decrease in the ECL intensity and the influence of other amino acids is negligible. This result confirmed that thiol plays a determinate role in the reaction between Cys and DBAEA. Therefore, this method shows good selectivity for Cys among other native amino acids.

3.9 The detection of Cys in real sample

As is well known, Cys is an essential nutrition that needs to be supplemented during the cell metabolism. To test the feasibility of this method, we detected Cys in Dulbecco's Modified Eagle Medium (DMEM) (without pretreatment except diluting for 10 times) using for the cultivation of Mouse embryonic fibroblasts (L929) under the optimized conditions. As shown in table 2, the concentration of Cys in DMEA was found as 0.210 mM. With standard addition method, the recoveries range from 93.5%-105.6, indicating the present method is feasible for real sample detection.

4. Conclusion

In this work, we synthesized DBAEA as a new co-reactant of $Ru(bpy)_3^{2+}$ through a one-step method. It can increase the ECL intensity dramatically on Pt electrode compared with conventional co-reactants. This method can be used to detect Cys quantitatively based on the conjugate addition/cyclization reaction between Cys and DBAEA, and it shows high selectivity and good sensitivity among other native amino acids. This work will broaden the ECL application by synthesizing new co-reactant from a design perspective and promote the sensitivity of analysis. It also shows great potential applications in biological detection, clinical analysis and environmental

monitoring.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgements

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Legends

Scheme 1. ECL detection of Cys based on the ECL of $Ru(bpy)_3^{2+}$/DBAEA on Pt electrode

Scheme 2. The synthesis method of DBAEA

Fig. 1. ECL intensity of 1 μM $Ru(bpy)_3^{2+}$/5 mM TPA (a) , 1 μM $Ru(bpy)_3^{2+}$/5 mM DBAE (b), 1 μM $Ru(bpy)_3^{2+}$/5 mM DBAEA (c) on Pt electrode (A), GCE (B) and Au electrode (C), pH = 7.5.

Fig. 2. CV curve of 1 μM $Ru(bpy)_3^{2+}$/5 mM TPA (a) , 1 μM $Ru(bpy)_3^{2+}$/5 mM DBAE (b), 1 μM $Ru(bpy)_3^{2+}$/5 mM DBAEA (c) on Pt electrode (A), GCE (B) and Au electrode (C), pH = 7.5.

Fig. 3. The dependence of ECL intensities on the concentrations of TPA, DBAE, DBAEA, C ($Ru(bpy)_3^{2+}$) = 1 μM, pH = 7.5, Pt electrode.

Scheme 3. ECL mechanism of $Ru(bpy)_3^{2+}$/DBAEA

Fig. 4. The ECL intensity (A) and CV curve (B) of 1 μM $Ru(bpy)_3^{2+}$ (a), 5 mM DBAEA (b), 1 μM $Ru(bpy)_3^{2+}$/5 mM DBAEA + 5 mM Cys (c) 1 μM $Ru(bpy)_3^{2+}$/5 mM DBAEA (d), 5 mM Cys (e), pH = 7.0, Pt electrode.

Scheme 4. The reaction mechanism of DBAEA and Cys.

Fig. 5. (A) The ECL decrease of $Ru(bpy)_3^{2+}$/DBAEA in the presence of different concentrations of Cys (10 μM, 30 μM, 50 μM, 70 μM, 0.1 mM, 0.2 mM, 0.3 mM, 0.5 mM, 1 mM, 3 mM, 5 mM, 10 mM, 30 mM, 50 mM and 70 mM). (B) The linear relationship of the logarithm of ECL decrease against the logarithm of C (Cys) from 10 μM to 200 μM. I_2 and I_1 represent the ECL intensity with and without Cys, separately, pH = 7.0, Pt electrode.

Fig. 6. The ECL intensity decrease of $Ru(bpy)_3^{2+}$/DBAEA in the presence of different

amino acids on Pt electrode. All the experiments were operated under the same condition: reaction solution: 50 μL amino acids (50 mM), 50 μL DBAEA (50 mM) and 150 μL pH 7.0 PBS; ECL solution: 200 μL pH 7.0 PBS + 50 μL $Ru(bpy)_3^{2+}$ (10 μM). I_2 and I_1 represent the ECL intensity before and after the reaction of DBAEA and amino acids.

Table 1. Comparison of different methods used for Cys detection.

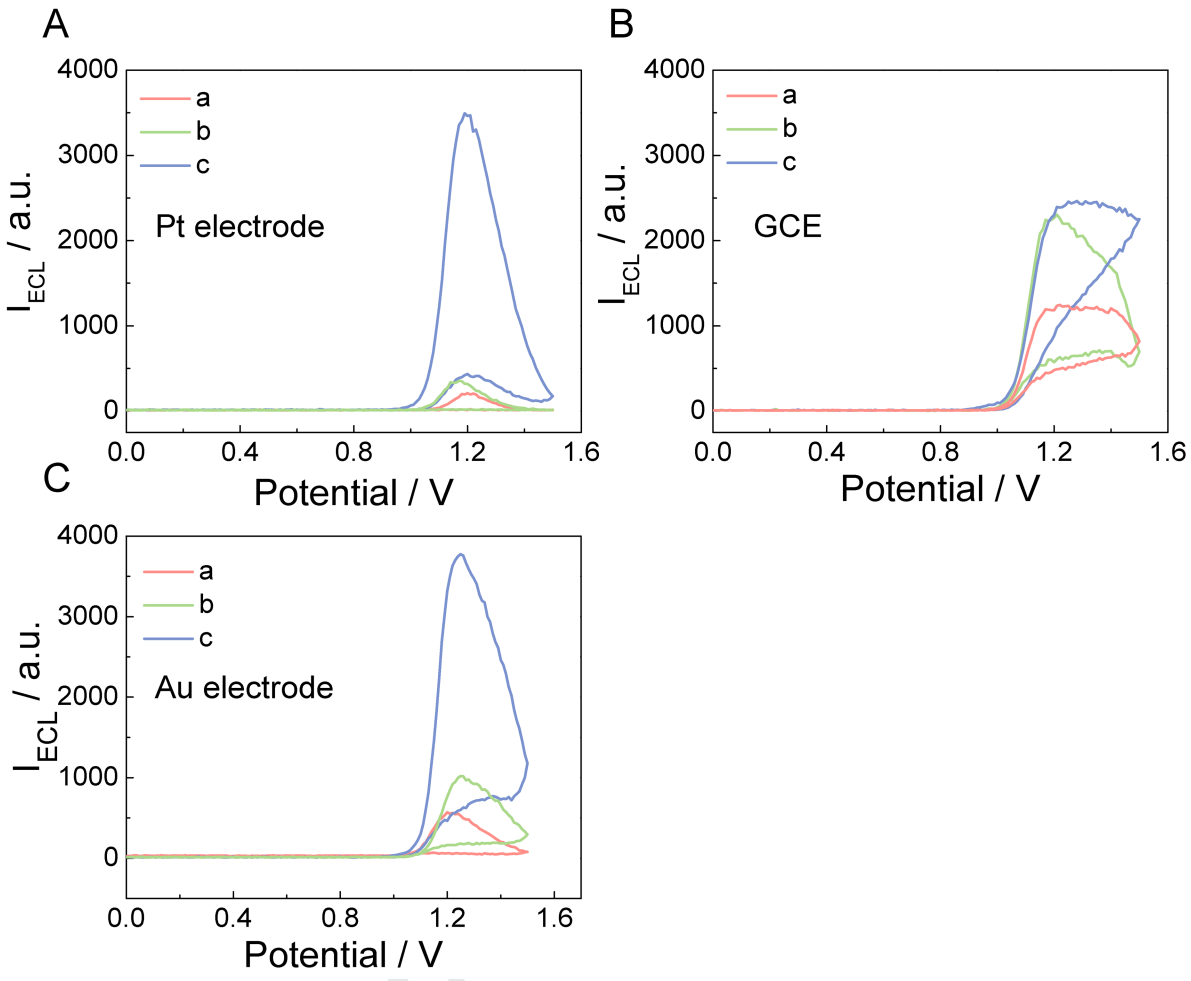
Table 2. The detection of Cys in DMEM

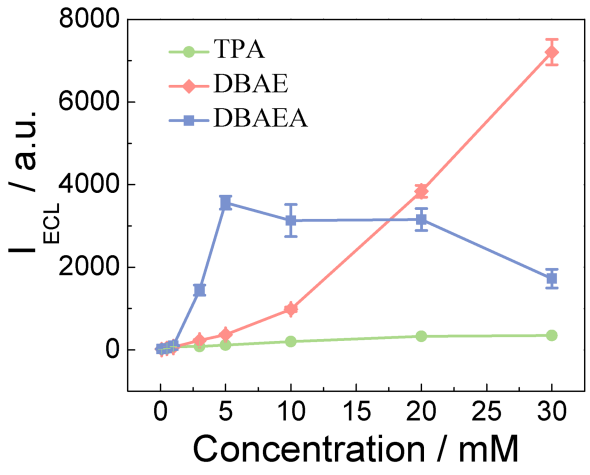
Table 1. Comparison of different methods used for Cys detection.

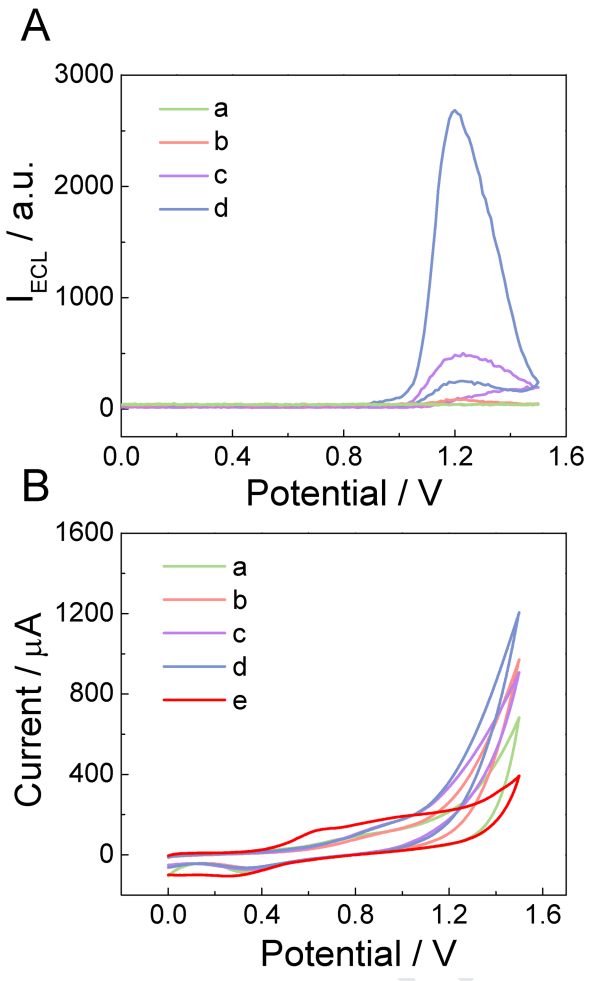
Method	**Probe**	**Linear range**	**LOD**	**Ref.**
Colorimetry	Au nanoparticles		1 μM	[27]
Fluorescence	Fluorogenic dye	100 μM-1.0 mM	39 μM	[28]
Electrochemistry	Pt/Fe_3O_4 nanoparticles	100 μM-1.0 mM	10 μM	[29]
Electrochemistry	Copper–cobalt hexacyanoferrate	6 μM-1.0 mM	5 μM	[30]
Fluorescence	Au(I) complex-based colloids	0-20 μM	1 μM	[31]
ECL	$Ru(bpy)_3^{2+}$/DBAEA	10 μM-2 mM	1.149 μM	This work

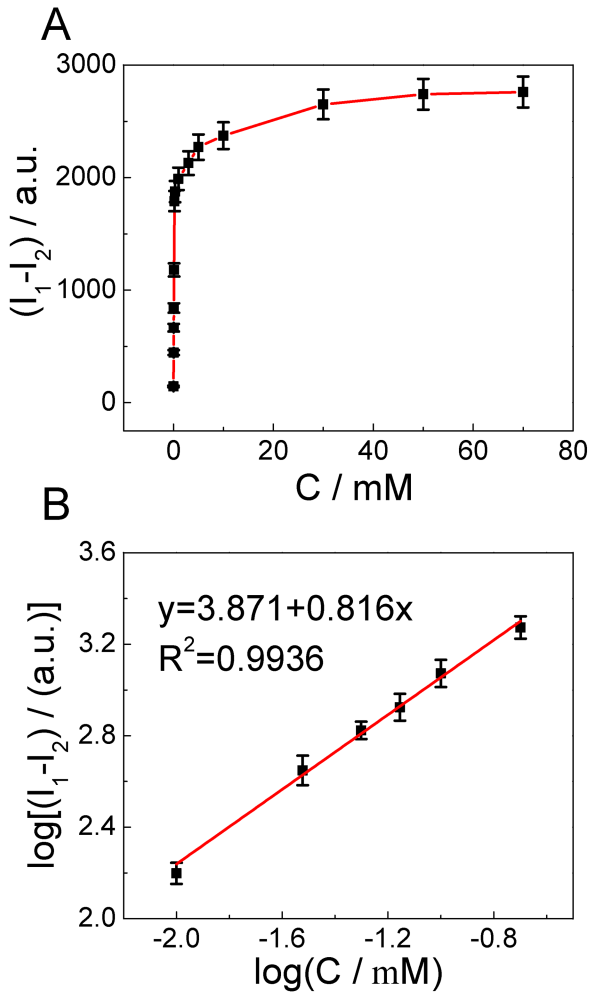
Table 2. The detection of Cys in DMEM

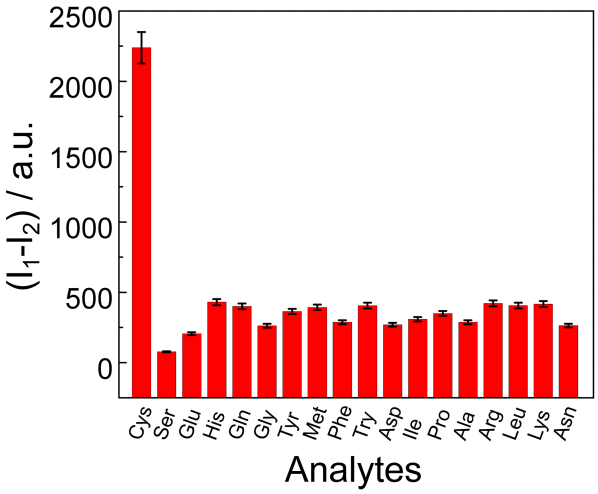
Cys added (mM)	Cys founded (mM)	RSD (%)	Recovery (%)
	0.210	4.54	
0.05	0.275	6.35	105.6
0.1	0.290	5.10	93.5
0.15	0.373	4.30	103.7

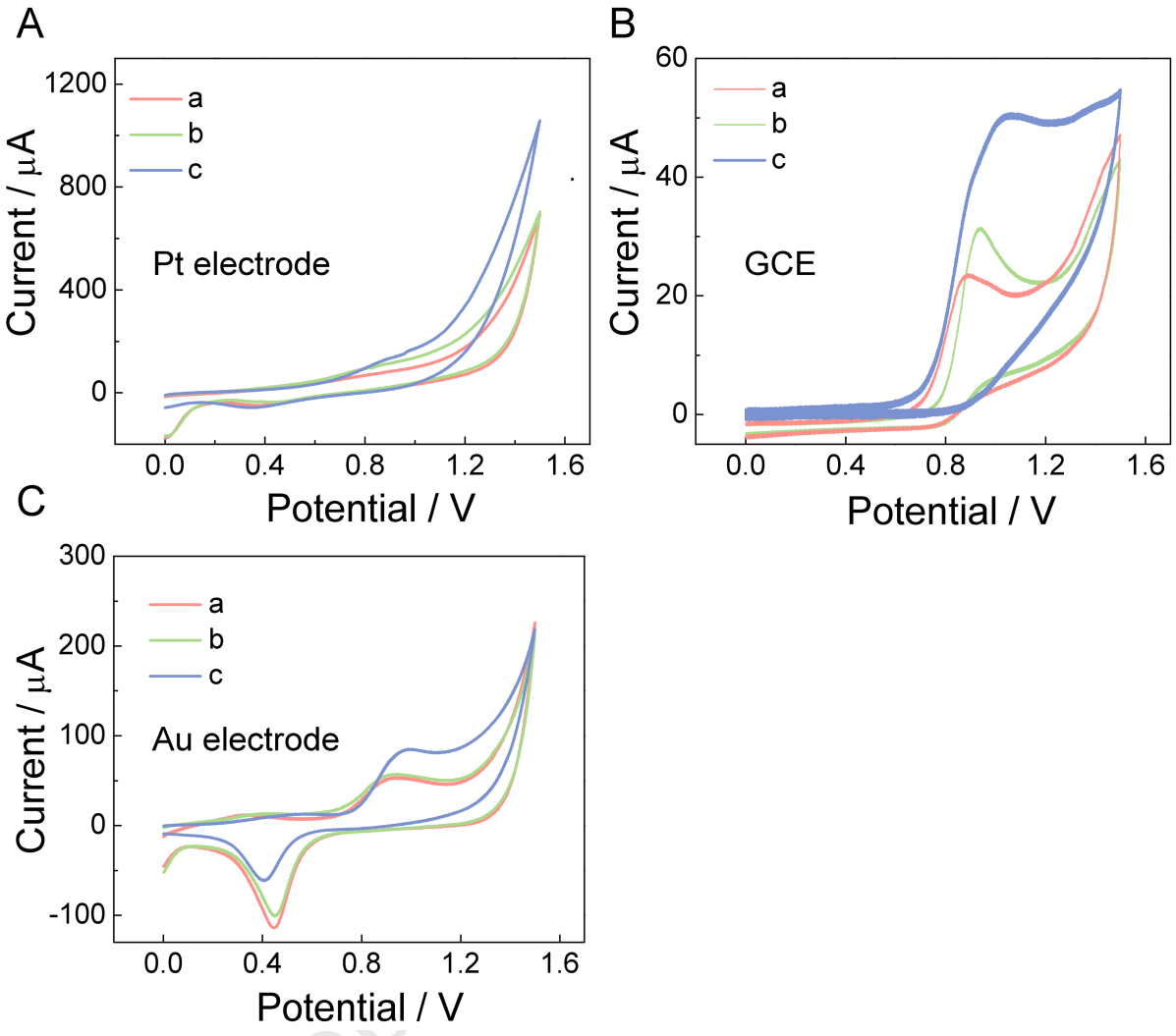

A
B
C
a
b
c
Pt electrode
GCE
Au electrode
I_{ECL} / a.u.
Potential / V
0
1000
2000
3000
4000
0.0
0.4
0.8
1.2
1.6

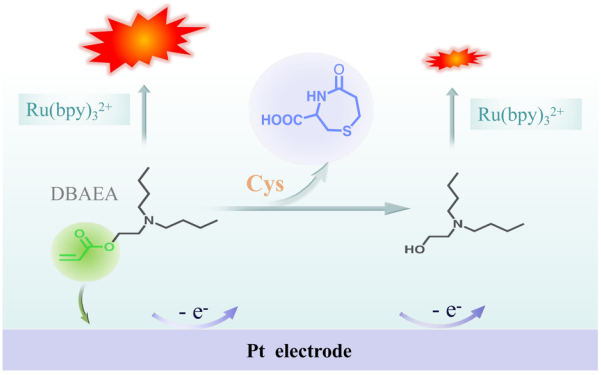


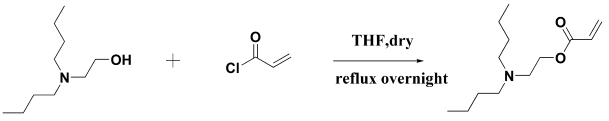


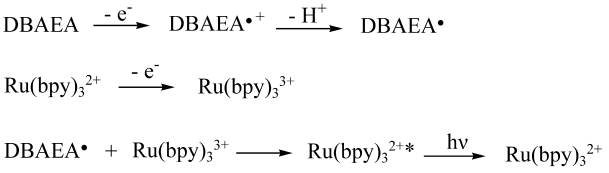


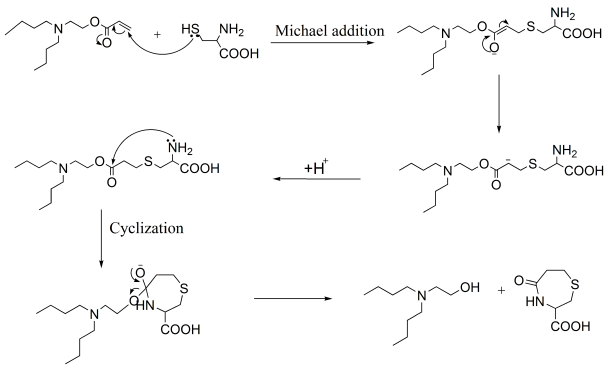



A
Current / μA
Potential / V
a
b
c
Pt electrode
B
GCE
C
Au electrode









Research Highlights

1. A new co-reactant DBAEA has been successfully synthesized.
2. The new co-reactant DBAEA has olefin group.
3. The olefin group can dramatically increase the ECL intensity of $Ru(bpy)_3^{2+}$.
4. Cysteine can remove the acrylate group of DBAEA selectively.
5. Cysteine can be detected selectively with $Ru(bpy)_3^{2+}$/DBAEA ECL system.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: