Development of electrochemiluminescent inhibition method for determination of gentian violet in aquatic water

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**A B S T R A C T**

Gentian violet (GV) was found to quench the electrochemiluminescence (ECL) of the tris(2,2’-bipyridyl)ruthenium(II)/tris-n-propylamine (Ru(bpy)32+-TPA) system at a glass carbon electrode (GCE). Based on the ECL signal changes, a simple and ultrasensitive detection method for GV in aquatic water was established. Under the optimized conditions, the quenched ECL intensity versus the logarithm of the concentration of GV was linear over a concentration range from 1.0 × 10−10 to 5.0 × 10−3 mol L−1, and the limit of detection (LOD) was found to be 4.5 × 10−15 mol L−1 (S/N = 3). The results obtained by the ECL system were better than other reported methods in literatures in terms of sensitivity or linear response range. The method was successfully applied to determine GV in aquatic water, and the relative standard deviations (RSDs) were found less than 6.3%, and the recoveries were obtained from 98.7 to 111.0%. Moreover, a possible mechanism of the quenching effect was primarily discussed based on UV–visible absorption spectra, cyclic voltammograms and I\(_{\text{ECL}}\)–E curves.

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1. Introduction

Gentian violet (GV, Scheme 1) belongs to the class of triarylmethane dyes, is also known as hexamethylpararosaniline chloride, basic violet 3, crystal violet, and methyl violet 10B. Its IUPAC name is N-[4-[bis(4-dimethylamino)-phenyl]-methylene]-2,5-cyclohexadien-1-ylidene]-N-methylmethanaminium chloride. GV is the active ingredient in Gram’s stain to classify bacteria and an acid–base indicator. The dye is used as an external skin disinfectant in humans and animals. However, GV dye is harmful by inhalation, ingestion, through skin contact. Some reports have linked long-term exposure to large amounts of GV with cancer and also been found to cause severe eye irritation in human beings [1–4].

Based on these researches, although GV was once introduced as an ectoparasiticide, fungicide and antiseptic in aquaculture [5,6], GV has been banned in several countries due to the risks associated now [7,8]. For example, it is not authorized by Food and Drug Administration (FDA), and it is restricted in the imports as an aquaculture veterinary drug in Japan and European [9], and it is also not permitted for use in China. However, it is still being used in many parts of the world due to their low cost, ready availability and efficiency. Thus, it is necessary to determine residues of GV in aquatic water or products.

Many methods for GV determination have been reported [8,11–21], mainly including chromatographic and spectroscopy detection. These methods usually require expensive instruments and cumbersome sample pretreatments; sometimes they have less sensitive and narrow linear response range, and suffer from some interference. Electrochemiluminescence (ECL) is a new analytical technique and has been widely studied, which is developed based on the combination of electrochemistry and chemiluminescence. It not only has the advantages of high sensitivity and selectivity, low detection limits, but also is easy to control the reaction of chemiluminescence by adjusting the potentials [22–24].

In reported ECL methods, Ru(bpy)32+ is one of the most popular electrochemiluminescence (ECL) systems. Based on ECL inhibition of ruthenium complexes, some sensitive methods were developed for the determination of phenol and substituted phenols [25], phenols and anilines [26], noradrenaline and dopamine [27], adrenaline [28], gallic acid [29] and chloride ions [30], etc. [31,32]. Up to now, Ru(bpy)32+-TPA ECL system is the most widely used [22–24]. In our work, the ECL signal of Ru(bpy)32+-TPA was obviously inhibited in the present of GV. Based on the ECL signal changes, a sensitivity ECL method for GV determination in aquatic water was developed. The possible inhibition mechanism for the ECL of Ru(bpy)32+-TPA by GV had been primarily studied. To our best knowledge, there is no report on GV determination by the ECL procedure of Ru(bpy)32+-TPA/GV system up to now.
2. Experimental

2.1. Reagents and apparatus

ECL intensity versus potential was detected by using a lab-made system, which consisted of a BPCL Ultra-Weak Chemiluminescence Analyzer (Institute of Biophysics, Chinese Academy of Sciences) and a CHI model 1100A electrochemical analyzer (Shanghai Chenghua Instrument Co., China). A conventional three-electrode system was used for the electrolytic system, including a glassy carbon electrode (GCE) as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl (sat. KCl) electrode as the reference electrode. A home made 5 mL cylindrical quartz cell was used as ECL cell, and was placed directly in the front of the photomultiplier tube. The ECL spectra of Ru(bpy)$_3^{2+}$, Ru(bpy)$_3^{2+}$/TPA system and Ru(bpy)$_3^{2+}$/TPA/GV system from 535 to 640 nm were obtained by putting a series of filters between the ECL cell and the photomultiplier tube one by one, and recording the ECL intensity successively. Results showed that maximum ECL intensity could be obtained at 620 nm. Hence, all the further experiments were tested by using the 620 nm filter. UV–visible absorption spectra were recorded with a Persee General (Beijing, China) TU-1901 spectrophotometer.

Ru(bpy)$_3^{2+}$/TPA and GV (purity, 99%) were obtained from Sigma Chemical Co. (USA) and used without further purification. Other chemical agents were analytical grade or better and double distilled water was used throughout. The stock solution concentration of Ru(bpy)$_3^{2+}$ is $1.0 \times 10^{-3}$ mol L$^{-1}$ and stored in the refrigerator. The stock standard solution was used to prepare working standard solutions daily by suitable dilution. The real samples were taken from the local aquatic ponds.

2.2. ECL procedures

For each experiment, the working electrode was polished consecutively with 1.0 µm, 0.3 µm and 0.05 µm α-Al$_2$O$_3$ aqueous slurries on a chamois leather to obtain a mirror surface. The electrode was then sonicated and thoroughly rinsed with deionized water, and was air dried before use.

1.0 mL sample solution and certain concentration Ru(bpy)$_3^{2+}$/TPA solution were added to a 10.0 mL volumetric flask, and diluted with buffer solution to the required volume, and 1.0 mL mixture solution was transferred to the ECL cell. A cyclic voltammetry was scanned in the range of +0.8–1.6 V with the scan rate of 0.05 V/s, and the ECL signal was then recorded. To eliminate the interference from dissolved oxygen, the sample solutions were degassed with argon for 15 min before taking measurements, and all experiments were done at room temperature (25 ± 1 °C) under argon.

Except for applications in Section 3.6, all the measurements were repeated at least three times. Some experiments were repeated five times or more. The repeat times depend on the repeatability and stability, which were based on the relative standard deviations (<5%).

3. Results and discussion

3.1. ECL behavior of Ru(bpy)$_3^{2+}$/TPA

Fig. 1 shows the cyclic voltammetry (CV) curves of bare GC electrode in three solutions under controlled conditions. The results showed that GV did not give ECL at GCE in the absence of Ru(bpy)$_3^{2+}$ (Fig. 1a). The ECL intensity of Ru(bpy)$_3^{2+}$/TPA at GCE in the presence of GV (Fig. 1c) was lower than that using Ru(bpy)$_3^{2+}$/TPA alone (Fig. 1b). The significantly decreased ECL signal indicates that GV is able to quench the ECL of Ru(bpy)$_3^{2+}$/TPA effectively.

3.2. The mode of applied potential and scan rate

The quenched ECL intensity ($\Delta I$) was correlated with a number of factors, including the scan mode and scan rate, the applied potential, the buffer solution and its pH condition. In order to obtain a higher sensitivity of ECL, all these factors were optimized.

Cyclic voltammetry (CV), linear sweep voltammetry (LSV) and differential pulse voltammetry (DPV) were used to examine the ECL behavior of Ru(bpy)$_3^{2+}$/TPA/GV system on a GC electrode with applied potential in the range of +0.8–1.6 V. The results showed that good and steady ECL signal (highest signal-noise-ratio in the ECL inhibition) was obtained using CV mode for the measurements. The effect of CV scan rates on the $\Delta I$ was also studied. $\Delta I$ increased gradually as the scan rate applied from 0.01 V/s to 0.05 V/s (Fig. 2). When the scan rate was >0.05 V/s, $\Delta I$ reached to a plateau. Therefore, a scan rate of 0.05 V/s was selected in the following experiments.

3.3. Effect of buffer solution and pH

The buffer solution has great effect on the ECL intensity of Ru(bpy)$_3^{2+}$/TPA. In this work, Britton–Robinson buffer solution,
NaHCO$_3$–Na$_2$CO$_3$ buffer solution, Tris–HCl buffer solution and phosphate buffer solution (PBS) have been tested. The results showed that the ECL intensity was stable and strong in phosphate buffer solution. Moreover, the effect of the pH value of the selected PBS buffer solution on ΔI has been studied also. As shown in Fig. 3, the ΔI reaches the maximum value at pH 8.0 Therefore, PBS buffer solution (pH 8.0) was chosen as the optimal condition for ECL experiments.

**Table 1**
Comparison of main methods reported for the determination of GV in aquatic products or real water samples.

<table>
<thead>
<tr>
<th>Analytical method</th>
<th>LOD or LOQ or LRR</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC–UV</td>
<td>0.5 μg L$^{-1}$ (LOD), 5.0–500 μg L$^{-1}$ (LRR)</td>
<td>[10]</td>
</tr>
<tr>
<td>HPLC-DAD</td>
<td>0.11–0.14 μg kg$^{-1}$ (LOD)</td>
<td>[16]</td>
</tr>
<tr>
<td>HPLC–ECD</td>
<td>0.5 ppb (LOD), 1–30 ppb (LRR)</td>
<td>[17]</td>
</tr>
<tr>
<td>HPLC–FD</td>
<td>0.5 μg kg$^{-1}$ (LOD)</td>
<td>[13]</td>
</tr>
<tr>
<td>HPLC–MS</td>
<td>0.2 μg kg$^{-1}$ (LOD), 0.5 μg kg$^{-1}$ (LOQ)</td>
<td>[14]</td>
</tr>
<tr>
<td>HPLC–ITMS</td>
<td>0.02–0.09 μg kg$^{-1}$ (LOD), 0.04–0.13 μg kg$^{-1}$ (LRR)</td>
<td>[19]</td>
</tr>
<tr>
<td>LC–VIS/MS</td>
<td>0.07–0.24 ng g$^{-1}$ (LOD)</td>
<td>[18]</td>
</tr>
<tr>
<td>CPE–UV/Vis</td>
<td>4.8 ng mL$^{-1}$ (LOD), 16–1000 ng mL$^{-1}$ (LRR)</td>
<td>[20]</td>
</tr>
<tr>
<td>MSPE–UV/Vis</td>
<td>0.5–1.0 mg L$^{-1}$ (LRR)</td>
<td>[8]</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>$1.58 \times 10^{-10}$ g mL$^{-1}$ (LOD), $1.0 \times 10^{-6}$ to $1.0 \times 10^{-1}$ g mL$^{-1}$ (LRR)</td>
<td>[12]</td>
</tr>
<tr>
<td>RRS</td>
<td>0.012 μg mL$^{-1}$ (LOD)</td>
<td>[11]</td>
</tr>
<tr>
<td>SERS</td>
<td>0.04–3.00 μg mL$^{-1}$ (LRR)</td>
<td>[15]</td>
</tr>
<tr>
<td>This method</td>
<td>$4.5 \times 10^{-12}$ mol L$^{-1}$ (LOD), $1.0 \times 10^{-10}$ to $5.0 \times 10^{-11}$ mol L$^{-1}$ (LRR)</td>
<td></td>
</tr>
</tbody>
</table>

3.4. Linear response range and detection limit

Under the above optimum conditions, the linear response range and the detection limit for GV determination was measured. The results showed that the quenched ECL intensity was found in good linear relationship with the logarithm of the concentration within the range of $1.0 \times 10^{-10}$ to $5.0 \times 10^{-7}$ mol L$^{-1}$ (Fig. 4), and the detection limit was $4.5 \times 10^{-12}$ mol L$^{-1}$. The regression equation was $\Delta I = 468.11 \log_{10} C + 513.88$ with a correlation coefficient of 0.9953, and the RSD $\times 0.005$.

The low detection limit indicates that this method is very sensitive for the determination of trace GV. The sensitivity of this method for the determination of GV in some dairy products or real water samples was listed in Table 1. The detection limits (LOD) or quantitation limits (LOQ) or linear response range (LRR) were also compared with other reported methods [8,10–20]. It can be found that the ECL method exhibits many advantages in the detection limit or other aspects [22–32], which is same as the most sensitive methods reported in literature.

3.5. Interference

In order to assess the proposed method for GV analysis in aquatic water, the interference effects of coexistence substances, which were expected to present in the aquatic water, were examined. The effect of foreign substances was tested by analyzing a standard solution of GV ($1.0 \times 10^{-7}$ mol L$^{-1}$) and interfering substances. The upper limit of an interfering species was estimated under the conditions, which the relative error for determination of a standard GV solution was $\pm 5\%$. The tolerable concentration ratios with respect to $1.0 \times 10^{-7}$ mol L$^{-1}$ GV for interference at 5% level were listed in Table 2. These results showed that this ECL method not only presented the better sensitivity but also offered high selective.

**Table 2**
The tolerable concentration ratios for some interfering species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tolerable concentration ratio$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$, K$^+$, NH$_4^+$, NO$_3^-$, Cl$^-$, SO$_4^{2-}$</td>
<td>1000</td>
</tr>
<tr>
<td>Ca$^{2+}$, Mg$^{2+}$, Zn$^{2+}$</td>
<td>300</td>
</tr>
<tr>
<td>Cu$^{2+}$, Fe$^{3+}$, Cd$^{2+}$</td>
<td>100</td>
</tr>
<tr>
<td>Malachite green and leucomalachite green</td>
<td>5</td>
</tr>
</tbody>
</table>

$^a$ Average values of five determinations.
Table 3
Determination of GV in aquatic water utilizing standard addition method.

<table>
<thead>
<tr>
<th>Added value (×10⁻⁹ mol L⁻¹)</th>
<th>Founded amount (×10⁻⁹ mol L⁻¹)</th>
<th>R.S.D. (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>0.5</td>
<td>0.53 ± 0.03</td>
<td>5.66</td>
<td>106.0</td>
</tr>
<tr>
<td>1.0</td>
<td>1.11 ± 0.07</td>
<td>6.30</td>
<td>111.0</td>
</tr>
<tr>
<td>10</td>
<td>9.87 ± 0.18</td>
<td>1.82</td>
<td>98.7</td>
</tr>
<tr>
<td>100</td>
<td>101.95 ± 2.96</td>
<td>2.90</td>
<td>102.0</td>
</tr>
</tbody>
</table>

a Average values of five determinations.
b No detection or below detection limit.

3.6. Applications

The real water sample was taken from a local fishing ground, and standing for 2 h at 6 ± 2 °C. Then 500 mL supernatant fluid was taken and filtered with 0.45 μm polytetrafluoroethylene (PTFE) micro- filtration membrane on standby. Prior to determination, the samples were treated according to the experimental section. As shown in Table 3, the proposed method was applied to detect the GV in aquatic water. The standard addition method showed that the average recoveries were range from 98.7 to 106.0%, and the relative standard deviations were less than 6.30%. Thus, this proposed method shows potential application for the determination of GV in aquatic water, and also in potable water or river ones.

3.7. Possible mechanism of ECL inhibition

The ECL mechanism of Ru(bpy)₃²⁺-TPA system have been thoroughly researched [22–24], which shows the reaction process between TPA free radical and Ru(bpy)₃²⁺ excitation state. In order to analyze the possible mechanism of ECL inhibition in the presence of GV, the UV–visible absorption spectra of Ru(bpy)₃²⁺/TPA/GV system were firstly obtained. The absorption maximum of GV ranges from 589 to 594 nm [1], and the maximum emission wavelength of Ru(bpy)₃²⁺ is about 620 nm [30]. As shown in Fig. 5, the absorption spectrum of the mixed system of Ru(bpy)₃²⁺-TPA/GV (Fig. 5c) was found exactly the same as the summation of Ru(bpy)₃²⁺-TPA (Fig. 5b) and GV (Fig. 5a). It strongly suggested that no new intermediate was produced as simply mixing the two compounds (Ru(bpy)₃²⁺-TPA/GV) in a solution. It is likely that there are other reactions.

From the cyclic voltammograms shown in Fig. 6, it could be found that oxidation peak of Ru(bpy)₃²⁺ at about +1.15 V (Fig. 6a) and peak of GV at about +0.9 V (Fig. 6b). It implied that the oxidation reaction of GV would simultaneously take place as the oxidation reaction of Ru(bpy)₃²⁺. Reference to the previous reports on the ECL enhancement mechanism of Ru(bpy)₃²⁺-TPA [22,30,32], there might be ECL enhancement performance between Ru(bpy)₃²⁺ and GV. Hence, IEC-E curves of Ru(bpy)₃²⁺/GV system were tested. The results showed that the ECL signal of Ru(bpy)₃²⁺** (Fig. 7a) could be enhanced by adding to GV, and the signal intensity would increase with GV concentration increasing (Fig. 7b and c). So, the radical quenching reaction between Ru(bpy)₃²⁺* and GV or GV would not happen, and the inhibition mechanism by GV absorbing electrochemiluminescence is also not reasonable. Additional explanation is necessary. Although GV could also enhance the ECL signal of Ru(bpy)₃²⁺*, its intensity is very weak comparing with Ru(bpy)₃²⁺-TPA system (Figs. 1 and 7). The weak signal strength is unfavorable for the sensitivity and detection limit in a quantitative analysis. Hence, this study finally adopted Ru(bpy)₃²⁺-TPA system instead of Ru(bpy)₃²⁺/GV system.

Based on our experimental results and ECL quenching mechanisms previously reported [30–34], a possible mechanism for the ECL inhibition of Ru(bpy)₃²⁺-TPA/GV system was proposed as shown in Fig. 8. The three compounds were oxidized on the electrode to produce their corresponding products. Then, the short-lived cationic radical TPA·⁺ is believed to lose a proton from an
R-carbon to form the strongly reducing intermediate TPA*. The TPA* is able to reduce Ru(bpy)_3^{2+} into Ru(bpy)_3^{3+}, which produces ECL emission.

Moreover, the result in Fig. 6 means that the oxidation reaction of GV would take place more easily as the oxidation reaction of Ru(bpy)_3^{2+}. Because the electrochemical reaction occurs only on the local surface of the electrode, the GV molecules would mostly changed into GV free radical by electro-oxidation process in the localized micro-space. In other words, the radical GV* form is main in localization surface of the electrode and the GV molecule form is main in solution matrix. So, although the concentrations of short-lived radical TPA* and GV* are very low in the overall system, the occurrence of the reaction between TPA* and GV* would be relatively easy in the high-concentration local surface. Consequently, the TPA* is consumed by the radical collisions between the product GV* and TPA*, and thus the ECL signal from Ru(bpy)_3^{2+} is quenched.

4. Conclusions

An inhibition ECL method based on the Ru(bpy)_3^{2+}-TPA for the determination of GV was successfully developed. It was simple, rapid, sensitive and wide linear range in comparison with the related literatures. It has good potential applications for the GV determination not only in real water samples but also in aquatic products. A possible mechanism was proposed for the explanation of the ECL inhibition behavior observed in this system. It was believed that the competition of the active free radicals between Ru(bpy)_3^{2+}-TPA and GV was the key factor for the ECL inhibition of the Ru(bpy)_3^{2+}-TPA.

Acknowledgments

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