Electrochimica Acta 53 (2008) 5451–5455

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

The potential-resolved electrochemiluminescent behavior of calcein in aqueous alkaline solutions at a glassy carbon electrode

Yinggui Zhu[a,b,*], Liusan Li[a], Hongling Qi[a], Boying Zhao[a], Guangchao Zhao[a,b,*]

[a] College of Chemistry and Materials Science, Anhui Normal University, Wuhu 241000, PR China
[b] Anhui Key Laboratory of Chemo-Biosensing, Wuhu 241000, PR China

ARTICLE INFO

Article history:
Received 31 December 2007
Received in revised form 17 February 2008
Accepted 18 February 2008
Available online 4 March 2008

Keywords:
Calcein
Potential-resolved
Electrochemiluminescence
Light emission
Cyclic voltammogram

ABSTRACT

In aqueous alkaline solutions, the potential-resolved electrochemiluminescent (ECL) behavior of calcein at a glassy carbon electrode was investigated. A sharp ECL light emission peak appeared near 0.8 V (vs. Ag/AgCl) under cyclic voltammogram (CV) conditions. In order to obtain a better understanding of the light emission, we investigated the effects of supporting electrolytes, the pH value, the presence of O_2 or N_2, and the concentration of calcein. The oxidative form of calcein reacted with hydroxyl radicals and oxygen, producing singlet excited oxygen that was identified as the emitter using the ECL, UV–vis absorption and fluorescence spectra. A possible mechanism for the behavior of calcein in this system was also proposed.

1. Introduction

Calcein (bis[*N*,*N*′-bis(carboxymethyl)aminomethyl]fluorescein), a common fluorescent reagent, has a similar backbone to fluorescein and is widely used in fluorescence analysis to determine the concentration of many metal ions and as an indicator for titration analysis [1,2]. The structures of calcein and fluorescein are shown in Fig. 1. The rapidly expanding literature in the field demonstrates that applications for calcein have recently attracted more and more attention [3–7].

Electrochemiluminescence (ECL), the production of light from electrically generated species at electrode surfaces [8], has received much attention during past 40–50 years since the first detailed studies in the mid-1960s. This is due to the versatility, simplified optical setup, and good temporal and spatial control of this method [9]. As a means of analytical detection, ECL has found many applications in basic science research and clinical analysis [10]. The electrochemiluminescent behaviors of various organic luminophors and their mechanisms have been previously reported, for example, those of lucigenin [11–13], luminol [14–16] and fluorescein [17,18]. To our knowledge, few previous papers have discussed the ECL of calcein. The current work presents the potential-resolved ECL behavior (i.e., the curves of ECL intensity (I) vs. potential (E) (I–E curves), comparable with cyclic voltammograms (CVs)) of calcein under cyclic voltammogram conditions in aqueous alkaline solutions at a glassy carbon electrode. We also propose a new ECL system for research into ECL as well as its practical application.

2. Experimental

2.1. Reagents

Bis[*N*,*N*′-bis(carboxymethyl)aminomethyl]fluorescein (calcein) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai). The 5.0×10^{-3} mol/L working solution of calcein was freshly prepared by dissolving 0.310 g of calcein into a solution of 1.0 mol/L NaOH and then diluting it to 100 mL with triple-distilled water (Shanghai Ya Rong triple-distilled water system). The buffer was composed of 0.0250 mol/L $Na_2B_4O_7$ solution and the pH was adjusted with concentrated NaOH. All other reagents used were of analytical grade. Nitrogen and oxygen (both at 99.999%) were used for the air-free experiments, and a constant flow of nitrogen and oxygen was maintained over the solutions during the measurements. Triple-distilled water was used throughout the whole experiment.

2.2. Apparatus and procedure

A CHI660C electrochemical analyzer (Chenhua Inc., Shanghai) was used for electrochemical experiments. A three-electrode cell

---

* Corresponding authors at: College of Chemistry and Materials Science, Anhui Normal University, No. 1 Beijing Road, Wuhu 241000, PR China.
Tel.: +86 5535670457; fax: +86 5533869303.
E-mail address: ygzhu08@mail.ahnu.edu.cn (Y. Zhu).

0013-4686/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.electacta.2008.02.078

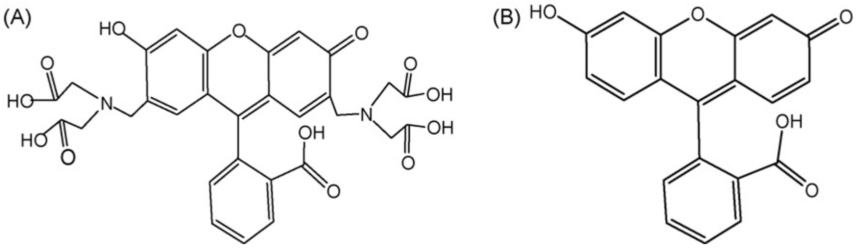

Fig. 1. Structures of calcein (A) and fluorescein (B).

was used, containing a 3.0 mm diameter GC disk electrode (working electrode), an Ag/AgCl/saturated KCl reference electrode, and a Pt wire that was separated from the working electrode with a glass capillary to avoid the background emission (counter electrode). ECL generation and detection were performed on a model MPI-E ECL analyzer system (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China). During detection, the voltage of the PMT was set at 800 V. A virgin glass vial (10 mm × 25 mm) housing the three electrodes (shown in Fig. 2) was used in each measurement. The cell was aligned with the photomultiplier tube of the ECL analyzer.

Before each experiment, the working electrode was polished with microfiber cloth with 0.05 μm γ-Al_2O_3 powder to obtain a mirror surface, and ultrasonicated in anhydrous ethanol, acetone, and triple-distilled water for 5 min each, and then rinsed by triple-distilled water. For electrochemical treatment, the working electrodes were subjected to repeat scanning in the potential ranges of −0.5 to 1.5 V (vs. Ag/AgCl) for several cycles in 0.10 mol/L PBS buffer until a reproducible voltammogram curve was obtained. Between samples, the working electrode was taken out of the ECL cell and treated with the above electrode-treatment procedures except without electrochemical treatment. This step was used to improve the reproducibility of the ECL measurements. All experiments were carried out at ambient room temperature (∼25 °C).

A model UV-3010 spectrophotometer (Hitachi, Japan) was used to obtain UV–vis absorption spectra. Fluorescence spectra were acquired on a model F-4500 photospectrometer (Hitachi, Japan), and the voltage of the PMT was set at 400 V. The ECL spectrum was obtained from a MPI-A multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remex Analytical Instrument Ltd. Co.).

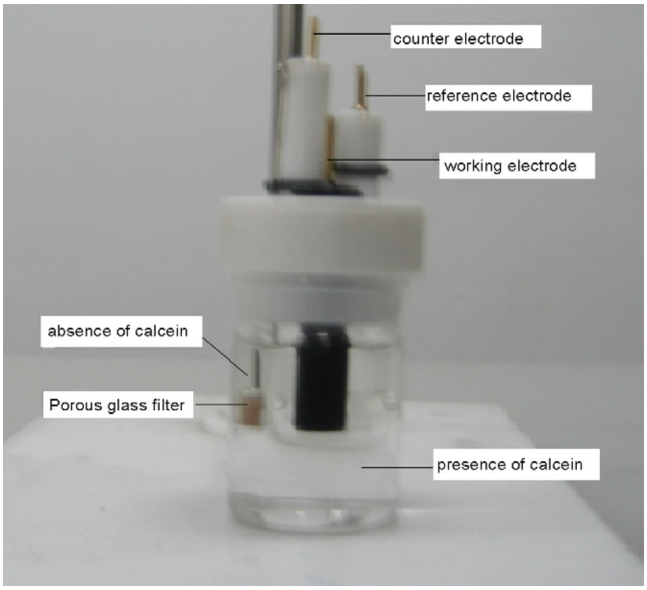

Fig. 2. Picture of the virgin glass vial housing the three electrodes.

3. Results and discussion

3.1. Electrochemistry and electrochemiluminescence of calcein in alkaline solution under an air-saturated atmosphere

Cyclic voltammograms and I_{ECL}–E curves of calcein in presence of 0.025 mol/L $Na_2B_4O_7$ (pH 10.0) and 0.01 mol/L Na_2SO_4 at a glassy carbon electrode under air-saturated conditions are presented in Fig. 3. We selected a scan range of −1.0 to 1.5 V, an initial potential 0.0 V, and a 0.1 V/s scan rate. Two obvious oxidative peaks were observed at about 0.46 V (cvp1, vs. Ag/AgCl) and 0.80 V (cvp2), while a weak oxidative peak appeared at 1.06 V (cvp3) on positive scan. cvp2 disappeared in the absence of calcein, implying that cvp2 correlated with the electrochemical oxidation behavior of calcein. This peak is similar to the oxidative peak at 0.94 V of fluorescein [17] in aqueous solution. cvp1 and cvp3 also appeared in 0.025 mol/L $Na_2B_4O_7$ + NaOH + 0.01 M Na_2SO_4 or 0.025 mol/L $Na_2B_4O_7$ + NaOH aqueous solutions without calcein, but cvp1 negative-shifted to about 0.40 V, indicating that cvp1 and cvp3 were not related to calcein. Previous studies [19,20] have suggested that the oxidation process of OH^- to O_2 included several steps, including:

$$OH^- \rightleftharpoons OH_{ads}{}^- \qquad (1)$$

$$OH^-{}_{ads} - e^- \rightleftharpoons OH_{ads} \qquad (2)$$

$$OH_{ads} \rightarrow\rightarrow O_2 \qquad (3)$$

cvp1 likely corresponded to step 2, and according to [21], cvp3 was likely due to the oxidation of OH^- to O_2, the electrode reaction, as follows:

$$OH^- - 4e^- \rightarrow O_2 + 4H_2O \qquad (4)$$

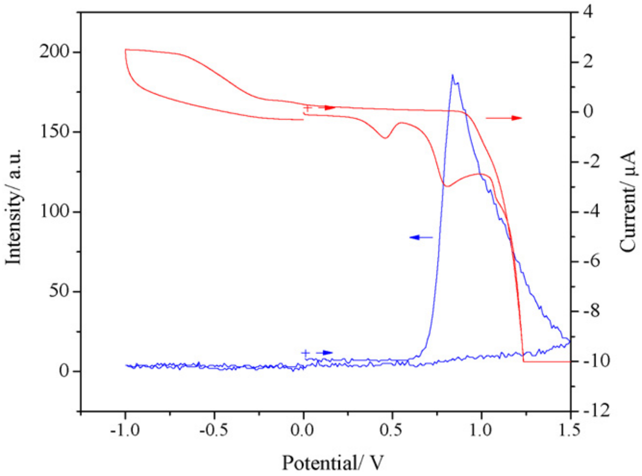

Fig. 3. CV and I_{ECL}–E curves of 5.0×10^{-4} mol/L calcein in 0.025 mol/L $Na_2B_4O_7$ (pH 10.0) and 0.010 mol/L Na_2SO_4 under an air-saturated atmosphere. The scan direction was positive and the scan rate was 0.1 V/s.