Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

The analytical determination and electrochemiluminescence behavior of amoxicillin

Li-Hua Shen [a,b,*], *Hong-Ni Wang* [b], *Pei-Jing Chen* [b], *Chun-Xia Yu* [b], *Yao-Dong Liang* [b], *Cheng-Xiao Zhang* [a,**]

[a] *School of Chemistry & Chemical Engineering, Shaanxi Normal University, Xi'an 710062, PR China*
[b] *School of Chemistry & Chemical Engineering, Xi'an University of Science & Technology, Xi'an 710054, PR China*

ARTICLE INFO

Article history:
Received 5 January 2015
Received in revised form
4 June 2015
Accepted 15 October 2015
Available online 22 December 2015

Keywords:
amoxicillin
electrochemiluminescence

ABSTRACT

A novel electrochemiluminescence (ECL) luminophor of amoxicillin was studied and found to generate ECL following the oxidation or reduction of amoxicillin. The amoxicillin oxidation state was also found to eliminate the reduction state, generating ECL. When solutions of amoxicillin were scanned between +1.5 V and −1.0 V with a graphite electrode in the presence of cetyltrimethyl ammonium bromide using KC1 as the supporting electrolyte, ECL emissions were observed at potentials of −0.7 V and +0.5 V. The ECL intensity at −0.7 V was enhanced by H_2O_2. Based on these findings, an ECL method for the determination of the amoxicillin concentration is proposed. The ECL intensities were linear with amoxicillin concentrations in the range of 1.8×10^{-8} g/mL to 2.5×10^{-7} g/mL, and the limit of detection (signal/noise = 3) was 5×10^{-9} g/mL. The florescence of amoxicillin had the greatest emission intensity in a neutral medium, with the emission wavelength dependent on the excitation wavelength. The experiments on the ECL mechanism for amoxicillin found that the electrochemical oxidation products of dissolved oxygen and active oxygen species contributed to the ECL process. The data also suggest that the hydroxyl group of amoxicillin contributed to its ECL emission.

1. Introduction

Amoxicillin (AM), a β-lactam antibiotic, is used to treat infections caused by certain bacteria, such as pneumonia, bronchitis, venereal disease, and ear, lung, nose, urinary tract, and skin infections. It may also be used before surgery or dental work to prevent infection [1,2]. The structure of AM is shown in Figure 1. The analytical methods used for the detection of AM in biological fluids are mainly based on high-performance liquid chromatography [3,4], spectrophotometry [5,6], capillary electrophoresis [7], fluorometry [8], and chemiluminescence [9–14].

* *Corresponding author*. School of Chemistry & Chemical Engineering, Xi'an University of Science & Technology, Xi'an 710054, PR China. Tel.: +86 29 85583183; fax: +86 29 85583182.
** *Corresponding author*. Tel./fax: +86 29 81530834.
E-mail addresses: Lihuashen1996@hotmail.com (L.-H. Shen), cxzhang@snnu.edu.cn (C.-X. Zhang).
http://dx.doi.org/10.1016/j.jfda.2015.09.002

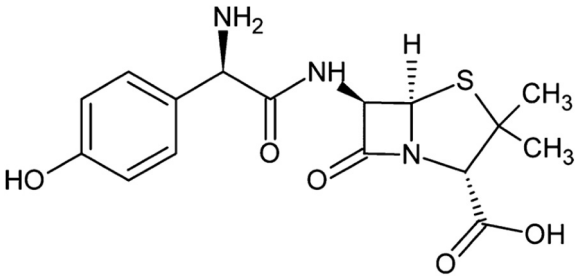

Fig. 1 – Molecular structure of amoxicillin.

Electrochemiluminescence (ECL) has emerged as a useful analytical technique in which luminescence is produced when an analyte is in the vicinity of an electrode surface with an applied potential [15]. Two commonly used ECL systems are the Ru(2,2-bipyridine)$_3^{2+}$ system and the luminol system. Both of these systems are usually applied in the positive potential region. However, the luminol system is known to suffer from interference by metal ions. The Ru(2,2-bipyridine)$_3^{2+}$ system is commonly operated at high potential (at or near +1.3 V), thus limiting its applicability. The ECL determination of β-lactamases employs the Ru(2,2-bipyridine)$_3^{2+}$ system [16], a system requiring the relatively expensive reagent Ru(2,2-bipyridine)$_3^{2+}$. Therefore, a new ECL system that does not employ this expensive reagent ECL would be of significant value. However, to date, only indole, tryptophan, and quercetin, which exhibit a different ECL behavior from luminol and Ru(2,2-bipyridine)$_3^{2+}$, have been reported [17,18]. A new ECL luminophor activated at a negative potential would represent a novel approach. To our knowledge, some quantum dots [19,20] and the Ru(2,2-bipyridine)$_3^{2+}$ system used in the presence of peroxysulfate can produce ECL emissions at negative potential [21]. This paper aims to use a fluorescent material that can be either oxidized or reduced electrochemically to generate ECL. Based on the ECL phenomena of the fluorescent material, we hope to develop a new ECL analytical method that widens the range of applications of ECL. The work reported in this paper demonstrates that electrochemical oxidization and reduction of amoxicillin generated ECL at a graphite electrode using a triangular pulse with KC1 as the supporting electrolyte. The intensity of the ECL was enhanced by both H_2O_2 and the surfactant hexadecyl trimethyl ammonium bromide. In addition, the oxidization state of amoxicillin eliminated its reduction state and generated ECL at a negative potential.

2. Methods

2.1. Reagents

All reagents were of analytical grade. Double-distilled water was used throughout the experiments. Amoxicillin was biochemical-reagent grade and was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (88%, Beijing, China). A stock solution of amoxicillin (1.00×10^{-3} g/mL) was prepared by dissolving 0.1136 g of amoxicillin in 10 mL of 0.10M hydrochloric acid and then diluting the resulting solution with water to a final volume of 100 mL. After the stock solution of amoxicillin had been stored for 1 week, the pH was adjusted to 7.0 using 0.1M NaOH. Standard working solutions were prepared daily by appropriate dilution of the stock solution with water. Additionally, 0.10M H_2O_2 and 1.25mM hexadecyl trimethyl ammonium bromide (CTAB) solutions were prepared as required. Amoxicillin capsules (Harbin General Pharmaceutical Factory, China) were purchased from a local hospital. Urine samples were collected from three healthy individuals from the Hospital of Shaanxi Normal University in Xi'an.

2.2. Apparatus

Cyclic voltammograms and the ECL signals produced in the electrolytic cell were detected and recorded using an MPI-Electrogenerated Chemiluminescence Analyzer (Xi'an Remax Analyse Instrument Co. Ltd., Xi'an China). The ECL measurements were performed using a three-electrode ECL system that included a graphite electrode as the working electrode ($\phi = 4.0$ mm), a platinum wire as the counter electrode, and an Ag/AgCl (Sat. KCl) electrode as the reference electrode. The ECL cell was placed directly in front of a photomultiplier tube (PMT) window and was enclosed in a light-tight box. The PMT was operated at −900 V, and the window was only opened towards the working electrode to eliminate background ECL from the counter-electrode. The working electrode was fixed on a Teflon cover to ensure reproducibility of the electrode position. All of the potentials were measured and reported relative to the reference electrode. UV–visible absorption spectra were measured with a TU-1901 Spectrophotometer (Beijing Currency Instrumental Co. Ltd., Beijing, China). The fluorescence spectra were measured with a Cary Eclipse Fluorescence Spectrometer (Varian, Palo Alto, CA, USA). The ECL spectra were measured using a Cary Eclipse Fluorescence Spectrometer by shielding the excitation optical window during constant potential scanning of the 2.5×10^{-4} g/mL AM/0.25M KCl/1.25mM CTAB/0.25M H_2O_2 at −0.7 V at the graphite electrode.

2.3. Procedure for ECL profiles and cyclic voltammograms

Each electrolytic cell contained a mixture of 1.0 mL of 1.0M KCl, 1.0 mL of 5.0×10^{-3}M CTAB, 1.0 mL of different concentrations of AM solution, and 1.0 mL of 1.0M H_2O_2. The ECL cell was placed in front of the PMT. Both ECL profiles versus potentials and the corresponding cyclic voltammograms were recorded simultaneously as the potential of the working electrode was scanned between +1.5 V and −1.0 V at a scan rate of 100 mV/s. When a potential of −0.7 V was applied at the graphite electrode, the ECL kinetic profiles were recorded. The ECL spectrum was measured using a Cary Eclipse fluorescence spectrometer while shielding the excitation optical window during the constant potential scanning of the 2.5×10^{-4} g/mL AM/0.25M KCl/1.25mM CTAB/0.25M H_2O_2 at −0.7 V at the graphite electrode.