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# Electrogenerated chemiluminescence for the sensitive detection of leucine using $Ru(bpy)_3^{2+}$ immobilized on dendritic Pd nanoparticle

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ABSTRACT

A novel electrogenerated chemiluminescence (ECL) sensor for the detection of amino acid based on the immobilized $Ru(bpy)_3^{2+}$ on dendritic Pd nanoparticle was firstly developed. The primary amino acid (leucine) was derivatized by reacting with acetaldehyde. The electrochemical and ECL behaviors of the immobilized $Ru(bpy)_3^{2+}$ were investigated. The modified electrode showed an increased electrocatalytic response to the oxidation of the derivatized leucine, producing a sensitized ECL signal probably since a high fraction of surface atoms located on the tips of the dendrites and a large amount of $Ru(bpy)_3^{2+}$ was immobilized on the electrode. Under optimal conditions, the sensor could be used for the determination of leucine with a linear range from 3.0 to 182 μM and a detection limit of 1.0 μM at 3$\sigma$. Furthermore, the present ECL sensor displayed a long-term stability. Our interference experiments indicated that acetaldehyde derivatives of other amino acids, such as proline and valine can be completely separated by electrophoresis (CE).



## 1. Introduction

Amino acids [1] have been analyzed for applications such as liquid chromatography [2,3], flow injection analysis [3,4] and electrogenerated chemiluminescence (ECL). ECL is the emission from an excited molecule generated by an electrochemical redox reaction. It has attracted much attention during past several decades due to its versatility, simplified optical setup, very low background signal and has become an important and valuable detection method in analytical chemistry [5,6]. It has been found in many applications, such as in immunoassays and DNA analyses [7], chemical sensing [8], imaging [9], lasing [10], and optical studies, and has been also used as detectors for chromatography and capillary electrophoresis. $Ru(bpy)_3^{2+}$ is one of the most extensively studied and used ECL compounds due to its superior properties including high sensitivity and stability under moderate conditions in aqueous solution, thus has been used for determination of a variety of amines containing analytes [11,12].

The $Ru(bpy)_3^{2+}$-based ECL intensity of the compounds containing the primary amine groups is very low. Furthermore, the electrogenerated $Ru(bpy)_3^{3+}$ can react with water to produce background ECL [13], which limits the detectability of amino amines, especially primary and secondary amines [14]. Thus, few works on the $Ru(bpy)_3^{2+}$-based ECL determination of amino acids have been reported except for proline [15–17]. The determination of primary amines can usually be improved through the structural change of analytes or derivatization with different reagents to convert primary amines to tertiary amines [18]. However, excess derivatization reagent or its disintegrated compound often interfere the detection due to the presence of the ECL signal from the derivatization reagents and the difficulty in separation. This work used acetaldehyde for the sensitive detection of primary amines (leucine). Acetaldehyde has been used as a derivatization reagent [19] and it could convert leucine to the corresponding derivative and it did not interfere with the determination of leucine since no ECL signal of acetaldehyde was detectable.

On the other hand, there is currently intense interest in the use of nanoparticles for the fabrication of modified electrodes and a wide range of bioscience applications [20,21]. Using nanoparticle to modify electrode surface has enhanced the response signal, increased the sensitivity, and showed better reproducibility. The hierarchical assembly of nanoscale building blocks into ordered superstructures or complex architectures, such as from one-dimensional nanorods to dendritic nanostructures and from dispersed nanocrystals to ordered network, may have many potential applications in the fields of catalysis, sensing, microelectronic devices (nanometer-scale electrodes), and electrochemistry, stemming from their special characteristics, such as numerous branches, the open channels between the branches, and large surface area, *etc.* Our previous report indicated that the biosensing properties based on the dendritic Pd were much better than those from spherical Pd nanoparticle [22].

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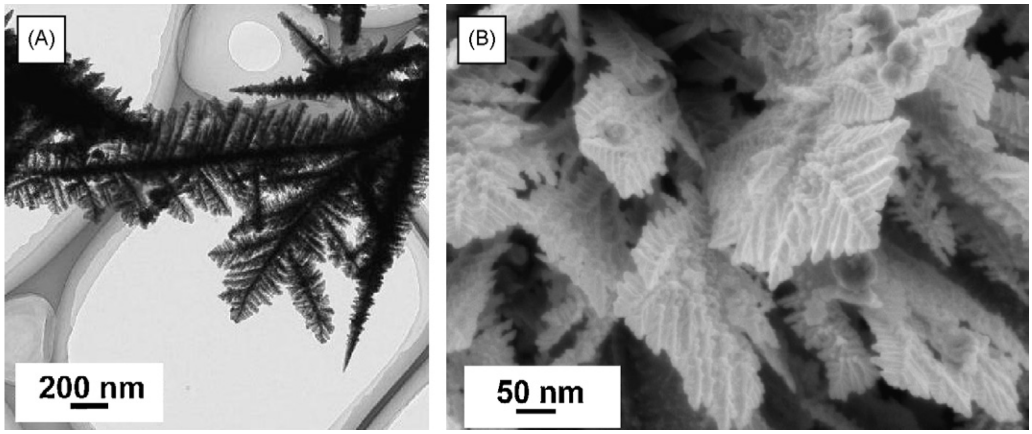


**Fig. 1.** TEM (A) and SEM (B) images of the Pd prepared by the typical reaction conditions.

To the best of our knowledge, however, the ECL behavior and the application of $Ru(bpy)_3{}^{2+}$ immobilized on Pd nanoparticle have not been reported yet. In this work, dendritic Pd nanoparticle was used to prepare ECL sensor for the first time by immobilizing $Ru(bpy)_3{}^{2+}$ on it. The modified electrode showed an electrocatalytic response to the oxidation of the derivatized leucine (d-leucine), producing a sensitized ECL signal. The reason of the enhanced sensitivity is probably that a large amount of $Ru(bpy)_3{}^{2+}$ was immobilized on the electrode and a high fraction of surface atoms located on the tips of the dendrites.

## 2. Experimental

### 2.1. Material

Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate (Ru (bpy)$_3$Cl$_2$·6H$_2$O) and leucine were purchased from Aldrich and used without further purification. Carbon graphite powder (<325 mesh) and paraffin oil were from Fluke. All other reagents were of analytical reagent grade. Solutions were prepared with twice-distilled water. Phosphate buffer solutions (PBS, 0.1 M) with various pH values were prepared by mixing stock standard solutions of $K_2HPO_4$ and $KH_2PO_4$ and adjusting the pH with $H_3PO_4$ or NaOH.

### 2.2. Preparation of dendritic Pd nanoparticle

Dendritic Pd nanoparticle was prepared following a recipe by our previous work [22]. In brief, in a typical synthesis, 20 mg of $PdCl_2$ was dissolved in 1.0 mL of concentrated HCl to make solution A. Then solution A, 33.3 mg $Na_4P_2O_7$ and 74 mg $NH_4F$ were added to 4.0 mL of 0.33 g mL$^{-1}$ polyglycol ($M_w$ 20,000) aqueous solution, followed by the addition of 25% ammonia. The pH value of the solution was then adjusted to 8.0 to obtain solution B. 1.0 mL of 80% hydrazine hydrate was added to 2 mL of 0.33 g mL$^{-1}$ polyglycol aqueous solution to make solution C. Next, solution B was added to solution C under sonication at 60 °C. After the obtained mixture was further sonicated for about 10 min, it was aged for 3 h and then separated by centrifugation. The deposit was washed with deionized water and ethanol several times. After vaccum drying, the black dendritic Pd was obtained. The $NH_4F$ and $Na_4P_2O_7$ used here acted as a ligand and a stabilizer of the solution, respectively [23].

### 2.3. Derivatization of amino acids

The derivatization of amino acids was followed as the literature [19]. A 50 μL aliquot of leucine was mixed with 5 μL of acetaldehyde, and then 10 μL of 0.2 M PBS (pH 7.0) was added. The final concentration of acetaldehyde was 0.4 mM. The mixture was allowed to stand for 1 h at room temperature.

### 2.4. Preparation of the modified electrode

The $Ru(bpy)_3{}^{2+}$–Pd nanoparticle modified carbon paste electrode (CPE) was prepared as follows. Prior to use, the graphite powder was treated at 700 °C for 30 s in a muffle furnace and then cooled to room temperature in a desiccator in the presence of activated silica gel. Ru(bpy)$_3$Cl$_2$·6H$_2$O was mixed with an equal molar Pd nanoparticle to obtain $Ru(bpy)_3{}^{2+}$–Pd. Then 100 mg of $Ru(bpy)_3{}^{2+}$–Pd were mixed with 200 mg of carbon graphite and 70 mg of the mixture and 25.2 μL of paraffin oil were mulled together by hand-mixing in order to obtain a uniformly wetted paste. A portion of the modified carbon paste was placed into the end of a Teflon tube (3 mm i.d.). Electrical contact to the paste was established by inserting a copper wire down the tubes and into the mixture. The Pd-CPE or ordinary CPE used for comparison was prepared in the same way by using Pd instead of $Ru(bpy)_3{}^{2+}$–Pd or omitting the $Ru(bpy)_3{}^{2+}$–Pd addition step. Prior to use the electrode tip was gently rubbed on a fine piece of paper to produce a flat surface.

### 2.5. Apparatus

The electrochemical measurements were carried out on a MPI-A multifunctional electrochemical analytical system (Xi'an Remex Electronic and Technological Co.) with a three-electrode system comprising platinum wire as the counter, Ag/AgCl (3.0 M NaCl)

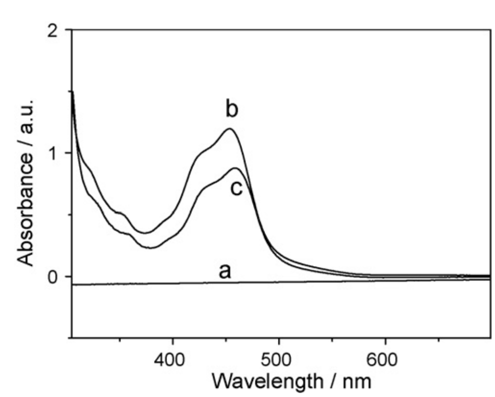


**Fig. 2.** Visible-region diffuse-reflectance spectra of Pd (a), $Ru(bpy)_3{}^{2+}$ (b) and $Ru(bpy)_3{}^{2+}$–Pd (c) nanoparticle.