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A novel electrochemiluminescence sensor coupled with capillary electrophoresis for simultaneous determination of quinapril hydrochloride and its metabolite quinaprilat hydrochloride in human plasma

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ARTICLE INFO

ABSTRACT

A novel electrochemiluminescence (ECL) sensor with composite consisted of silica-sol, Zinc oxide nanoparticles (ZnO NPs), polyvinylpyrrolidone (PVP) and tris(2, 2′-bipyridine) ruthenium (II) was constructed. A new method for simultaneous determination of quinapril hydrochloride (QHCl) and its metabolite quinaprilat hydrochloride (QTHCl) in human plasma was developed using the ECL sensor coupled with capillary electrophoresis (CE). ECL intensities of QHCl and QTHCl increased dramatically when the ECL sensor was used as working electrode. The running buffer contains 14 mmol/L phosphate (pH 8.0) and 20% n-propyl alcohol. Under optimized experimental conditions, the linearity ranges of the method are 0.007–8.0 µg/mL for QHCl and 0.009–8.3 µg/mL for QTHCl. The detection limits of QHCl and QTHCl (S/N = 3) are 3.6 ng/mL and 3.9 ng/mL, respectively. The method was applied for the simultaneous determination of QHCl and QTHCl in human plasma with satisfactory results.

1. Introduction

In ECL study, ECL sensors were constructed based on ECL reagent immobilized on the electrode. It did not only reduce the luminous reagent consumption and analysis cost, but also can simplify experimental design [1,2]. Nano-particles can significantly improve the determination sensitivity of ECL sensors due to their unique electrical and optical properties [3]. At present, nano-modified ECL sensors have become a hot topic of ECL research [4–8]. Zinc oxide nanoparticles (ZnO NPs) were applied on photoelectro-catalysis [9], gas sensor [10], modified electrode due to their good physic properties, such as good electrical conduction, catalytic activity, optical performance, and adsorption ability [11–13].

In recent years, the application of sol-gel technology has gradually increased in electrochemistry analysis, especially in the preparation of composite electrode and film modified electrodes because of its good characteristics in conductivity, photovoltaic cells and electrocatalysis [14–16]. Silica-sol is a translucent colloidal solution and could form a three-dimensional network structure which has the properties of good biocompatibility, physical rigidity, chemically inertness, thermal stability and almost no swelling properties in water [14]. The applications of silica sol-gel technology in preparing modified electrode had been

reported recently [17–21]. Silica-sol has film-forming properties. However, the existence of high shrinkage in the film-forming process could result in membrane cracks easily. Therefore, the water-soluble organic polymer can be added as the auxiliary film. Poly-vinylpyrrolidone (PVP) is a kind of water-soluble amino polymer with flexible chain structure, which provided with high solubility, film-formation, complexation property and surface activity can be used as coating agent, dispersant, thickener, and binder, etc. [14]. Quinapril (Fig. S1A) hydrochloride (QHCl) is an active angiotensin converting enzyme inhibitor used as a safe and high-performance antihypertensive drug in treating hypertension and heart failure [22,23]. Its active metabolite, quinaprilat (Fig. S1B) hydrochloride (QTHCl) showed higher angiotensin converting enzyme activity on heart, arteries and kidney than QHCl used as angiotensin converting enzyme inhibitor [24,25]. Until now, some methods have been developed for the simultaneous determinations of QHCl and QTHCl, including high performance liquid chromatography (HPLC) coupled with UV detection [26], solid phase extraction HPLC method [27], ultra-performance liquid chromatography–electrospray ionization mass spectrometry (UHPLC-ES-MS/MS) [28], HPLC-MS/MS [29]. These methods have drawbacks, such as costly instrumentation, large reagent use and time-consuming procedures. Capillary electrophoresis with electrochemiluminescence (CE-

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ECL) possesses the advantages of high sensitivity, wide linearity range, less sample volume, good selectivity, and low equipment cost. It has been widely applied to pharmaceutical analysis in recent years [30–33]. However, CE-ECL for the simultaneous determination of QHCl and QTHCl has not been reported. In this paper, a silica-sol/ZnO NPs/PVP/Ru(bpy)$_3$$^{2+}$ ECL sensor was prepared for the simultaneous determination of QHCl and QTHCl based on CE separation. ZnO NPs were applied for enhancing electron translation rate and adsorption ability of Ru(bpy)$_3$$^{2+}$. Silica-sol was used to fix Ru(bpy)$_3$$^{2+}$ on the electrode surface. PVP was added into silica-sol to prevent the film rupture. The electrochemical properties and detection performances of the sensor were investigated in detail. The method selectivity was greatly improved due to CE powerful separation.

2. Experimental

2.1. Apparatus and reagents

The MPI-B CE–ECL system was produced by Xi'an Remex Electronic Science-Tech Co., Ltd. (Xi'an, China). It consists of four main parts: a numerical control capillary electrophoresis high-voltage power supply, a multifunction chemiluminescence detector, a multichannel data collection analyzer and a numerical control flow injection sample injector [34–36]. To meet the needs of the measurement, an end-column ECL cell designed in reference was used and it is composed of a three-electrode system: a silica-sol/PVP/ZnO NPs/Ru(bpy)$_3$$^{2+}$ sensor as the working electrode, a Pt wire as the auxiliary electrode and Ag/AgCl (saturated KCl) as the reference electrode [37]. A 50 cm length of uncoated fused-silica capillary (75 μm i.d.) was obtained from Yongnian Optical Conductive Fiber Plant (Hebei, China). A Model HSJ-4A pH meter was produced by Shanghai Precision and Scientific Instrument Corporation (Shanghai, China). A XW-80A vortex mixer was obtained from Haimen Kylin-Bell Lab Instruments Co., Ltd. (Jiangsu, China). JEM-2100F transmission electron microscope (JEOL, Tokyo, *Japan*); Tris(2,2′-bipyridyl) ruthenium(II) chloride hexahydrate was purchased from Alfa Aesar (Johnson Matthey, Ward Hill, MA, USA). QHCl was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) and QTHCl was produced by Shanghai Zhenzhun Biological Technology Co., Ltd. (Shanghai, China). QHCl tablets were produced by Harbin Pharmaceutical Group Co., Ltd. (Harbin, China); NH$_4$HCO$_3$ was produced Tianjin Guangfu Technology Development Co., Ltd. (Tianjin, China); ZnSO$_4$, Na$_2$HPO$_4$, Na$_3$PO$_4$, NaH$_2$PO$_4$, NaOH, Tween 80 (TW-80), n-propyl alcohol were purchased from Xilong Chemical Co., Ltd. (Guangdong, China). Silica-sol was produced by Jining Huide Chemical Co., Ltd. (Jining, China). Polyvinylpyrrolidone (PVP), β-cyclodextrin (β-CD) and sodium dodecyl sulfate (SDS) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents used were of analytical grade. Double-distilled water (DDW) was used throughout.

2.2. Sample preparation

3.0 mL of blank blood sample was drawn from a healthy adult male volunteer with weight 62 kg and height 172 cm. The volunteer was not taking any medications in the past month. QHCl tablets (containing 20 mg QHCl)) were taken with 250 mL lukewarm water, and then 3.0 mL blood sample was obtained from elbow vein after 1 h. The blood samples were extracted according to the reference [38]. Firstly, they were set in centrifugal test tubes which were treated by heparin sodium, then centrifuged at 3500 rpm for 10 min in order to separate the plasma. 200 μL plasma was accurately transferred into a centrifugal tube, then 50 μL of 0.10 mol/L NaOH and 2 mL ethyl acetate were added into the centrifugal tube. After being shaken on a vortex mixer for 5 min, the mixtures were centrifuged at 12,000 rpm for 10 min. The upper layer was transferred into another tube and the lower residue was extracted twice again with ethyl acetate according to the previous step,

then merged the ethyl acetate extracts and evaporated to dryness under a stream of dry nitrogen at 80 °C. The dry residue was dissolved in 200 μL double distilled water (DDW). Prior to analysis, sample solutions were filtered through 0.45 μm membrane filters.

2.3. Preparation of ZnO NPs

ZnO NPs were prepared according to reference and some improvements were made [39]. Under the rapid magnetic stirring, 2.0 mL NaOH solution (0.5 mol/L) was dropwise added into 50 mL ZnSO$_4$ solution (0.10 mol/L). Continued to stir for 0.5 h and formed a translucent gel-like solution, which was alkaline zinc carbonate precursor solution. ZnO NPs were obtained by solid state reaction after the precursor solution being dried at 80 °C, then roasted at 300 °C for 2 h. ZnO nanoparticles were dispersed in 5 mL DDW by using ultrasonic waves and were purified by dialysis for 12 h using dialysis bag with molecular weight cut off 6000–8000 in DDW. Dried the purified ZnO NPs solution at 60 °C and pure ZnO NPs with particle size about 20 nm were obtained.

2.4. Preparation of modified sensor

The glassy carbon electrode (GCE) was treated by being polished with 0.30 μm and 0.050 μm Al$_2$O$_3$ polishing powder and cleaned by ultrasound in a mixed solution of ethanol and DDW with the volume ratio of 1:1, then waited to dry naturally. Added 1.5 mg ZnO NPs into 0.3 mL silica-sol and mixed it with ultrasound for 30 min, then added 0.15 mL PVP (0.05%) and 0.15 mL Ru(bpy)$_3$$^{2+}$ (2.0 mmol/L), continued to mix with ultrasound for 30 min and obtained a silica-sol/PVP/ZnO NPs/ Ru(bpy)$_3$$^{2+}$ compound solution. Immersed the processed glassy carbon electrode in the compound solution for 30 s, then took out and placed it at 4 °C for 12 h due to gaining stable sensor. After being washed three times by DDW, the silica-sol/PVP/ZnO NPs/Ru(bpy)$_3$$^{2+}$ sensor was prepared for experiment. The schematic fabrication process of ECL sensor and CE-ECL detection was shown in Fig. 1.

2.5. Procedures

A new capillary was activated by 0.1 mol/L NaOH for 12 h. Before each run, the activated capillary was flushed with 0.1 mol/L NaOH for 10 min and DDW for 10 min, and then the corresponding running buffer for 10 min. Adjust the distance of silica-sol/PVP/ZnO NPs/Ru(bpy)$_3$$^{2+}$ sensor and capillary outlet end about 200 μm. A 350 μL 55 mmol/L (pH = 8.0) phosphate solution was added into the ECL detection cell before the experiment. In all experiments, samples were introduced into the capillary by electrokinetic injection at 12 kV for 10 s and separated in the capillary at 12 kV. Detection potential was fixed at 1.20 V. The running buffer (pH 8.0) contained 14 mmol/L phosphate and 20% n-propyl alcohol. The potential of the photomultiplier tube (PMT) was operated at 800 V.

3. Results and discussion

3.1. Effect of modification agents on ECL intensity

The prepared ZnO NPs were characterized by transmission electron microscopy. As shown in Fig. 2A, the particle size was homogeneous and it was about 20 nm The XRD patterns of the prepared ZnO NPs were shown in Fig. 2B. All the detectable peaks could be indexed as the ZnO hexagonal wurtzite structure found in the standard reference data (JCPDS: 36e1451). Effect of modification agent on ECL intensity was investigated. The dosage of different reagents in modifier has great influence on ECL intensity. When silica-sol volume was 0.3 mL, the effects of ZnO NPs, PVP and Ru(bpy)$_3$$^{2+}$ amount on ECL intensity were studied in the paper. The ECL intensity obviously increased with the increasing of ZnO NPs use level and reached the maximum when ZnO