Journal of Electroanalytical Chemistry 712 (2014) 139–145

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

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

Determination of ascorbic acid in individual liver cancer cells by capillary electrophoresis with a platinum nanoparticles modified electrode

Xiaolei Wang *, Lingjun Li, Zhongyi Li, Jun Wang, Hongyan Fu, Zhenzhen Chen *

College of Chemistry, Chemical Engineering and Materials Science, Key Lab of Molecular and Nano Probes, Engineering Research Center of Pesticide and Medicine Intermediate Clean Production, Ministry of Education, Shandong Provincial Key Laboratory of Clean Production of Fine Chemicals, Shandong Normal University, Jinan 250014, PR China

ARTICLE INFO

Article history:
Received 1 August 2013
Received in revised form 1 November 2013
Accepted 11 November 2013
Available online 23 November 2013

Keywords:
Platinum nanoparticles
Chemical modified electrode
Capillary electrophoresis
Ascorbic acid
Single-cell analysis

ABSTRACT

Nanoparticles-based electrochemical sensor has received significant interest. In this work, platinum nanoparticles modified carbon fiber micro-disk electrode (PtNPsME) was electrochemically prepared and exploited as an amperometric detector for capillary electrophoresis. The prepared sensor displayed rapid and sensitive response towards ascorbic acid (AA), which was attributed to the electrocatalytic effect of platinum nanoparticles. Under optimized detection conditions, AA responded linearly from the range of 1.0 μM to 8.0 μM and 8.0 μM to 1 mM with correlation coefficients of 0.9981 and 0.9993. The concentration detection limits was 0.5 μM (S/N = 3). Compared with carbon fiber micro-disk electrode, the sensitivity was enhanced nearly four times. Acceptable repeatability of the microanalysis system was verified by ten consecutive injections of 0.5 mM, 50 μM and 5 μM AA without capillary and electrode treatments, the relative standard deviations of peak areas and migration time were 4.9% and 2.5%; 5.3% and 2.5%; 5.9% and 2.8%, accordingly. Meanwhile, well-shaped electrophoretic peaks were observed, mainly due to the fast electron transfer of electroactive species on the PtNPsME. The developed capillary electrophoresis–electrochemistry setup was first applied to the determination of ascorbic acid in single liver cancer cells (HepG2). The mean amount of AA in HepG2 cells extract and the average amount in single cells could amount to 9.4 ± 1.3 fmol (n = 3) and 12.9 ± 4.3 fmol (n = 20), respectively. Several merits of the novel electrochemical sensor coupled with capillary electrophorsis, such as comparative repeatability, easy fabrication and high sensitivity, hold great potential for the single-cell assay.

1. Introduction

Chemically modified electrodes (CMEs) have explored widely in the field of electroanalytical chemistry to date [1]. In the past decade, nanomaterials have attracted vast concerns for the preparation of electrochemical sensors, in which nanoparticles (NPs) have been researched more intensively for electrochemistry and biosensor applications [2–5]. Due to their small size, NPs exhibit unique physical, chemical and electronic properties. Furthermore, metal nanoparticles show excellent conductivity and catalytic properties [6]. Platinum nanoparticles (PtNPs) are extensively used as electrocatalysts in electrochemical applications, attributed to their high reactivity, high electrocatalytic efficiency, chemical inertness and high selectivity [2]. PtNPs have been widely applied to constructing electrochemical biosensors and chemically modified electrodes. For instance, PtNPs modified electrodes are fabricated to detect various analytes, including hydrogen [2], glucose [5], hydrogen peroxide [7], cholesterol [8], ascorbic acid, dopamine and uric acid [9] and so on.

Ascorbic acid (AA), vitamin C, is an essential nutrient for mammalian cells, acting as a cofactor of different enzymatic reactions, e.g. collagen synthesis. AA can mitigate the reactive oxygen species production triggered by lipopolysaccharide and thereby prevent the induction of nitric oxide synthase and excessive production of nitric oxide that worsens oxidative stress in hepatocytes [10]. Additionally, it is reported that AA can suppress the proliferation and metastasis of liver cancer cells possibly with a mechanism

Abbreviations: AA, ascorbic acid; CMEs, chemically modified electrodes; NPs, nanoparticles; CE, capillary electrophoresis; ECD, electrochemical detection; EP, epinephrine; Trp, tryptophan; UA, dopamine; Cys, L-cysteine; UA, uric acid; PB, phosphate buffer solution; PBS, balanced phosphate buffered saline; SCE, saturated calomel electrode; PtNPsME, platinum nanoparticles modified carbon fiber micro-disk electrode; CFME, carbon fiber micro-disk electrode; i_p, peak current; $W_{1/2}$, peak half-height; C_b, running buffer concentration; t_m, migration time; N, theoretical plate number.

* Corresponding authors. Tel./fax: +86 531 82615258 (X. Wang). Tel.: +86 531 86182545 (X. Chen).

E-mail addresses: wangxl@sdnu.edu.cn (X. Wang), zzchen@sdnu.edu.cn (Z. Chen).

1572-6657/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jelechem.2013.11.010

associated with the scavenging of reactive oxygen species [11]. Determing AA at single-cell level can reveal the difference of AA between cells and help to study the primary function of in both plants and animals.

Up to now, various analytical methods have been employed to study AA including chemiluminescence [12], high performance liquid chromatography [13], fluorimetry [14], capillary electrophoresis (CE) [15] and electroanalysis [16]. Electrochemistry (especially amperometric detection) integrated with separation techniques, like liquid chromatography, conventional capillary electrophoresis and microchip capillary electrophoresis, has gained remarkable advancement [17]. CE with electrochemical detection (ECD) can typically detect analytes present at femto- to attomole levels. CE–ECD has already been applied to analyzing single cells because it can offer high sensitivity and selectivity for electroactive analytes [18,19]. It has been used to determine AA in single cells, such as isolated pea plant cells [20], single human neutrophils [21] and single rat peritoneal mast cells [22] by carbon fiber micro-disk electrodes. Developments of microelectrodes fabrication techniques and different electrode materials can improve both sensitivity and selectivity, which facilitates the widespread application of electrochemical detection in single-cell analysis.

This work is aimed at evaluating the utility of PtNPs modified carbon fiber micro-disk electrode (PtNPsME) as electrochemical detector on capillary electrophoresis platform. Pt nanoparticles were electrodeposited onto the carbon fiber micro-disk electrode. The electrocatalytic activity of the modified electrode for ascorbic acid was characterized by cyclic voltammetry. Compared with traditional electrodes, PtNPsME provided an enhanced effective electrode surface and high catalytic activity toward AA. The present electrochemical sensor exhibited sensitive and rapid response to AA. The prepared electrode was then applied to capillary electrophoresis for electrochemical detection. This research, to our knowledge, is reported for the first time on such a PtNPsME assembly on CE for the measurement of AA.

2. Experimental

2.1. Reagents and apparatus

All reagents were of analytical grade and used as received otherwise specified statement. AA, epinephrine (Ep), tryptophan (Trp) and dopamine (DA) were purchased from Sigma Chemical Company (USA). L-cysteine (Cys) and uric acid (UA) was from Amresco (USA). H_2PtCl_4 was obtained from China Pharmaceutical Group Shanghai Chemical Reagent Company. The deaerated stock solution of 0.1 M AA was prepared with doubly distilled water weekly and was stored at 4 °C. The running buffer was 25 mM pH 7.4 phosphate buffer solution (PB). Various concentrations of the AA solutions were prepared by a serial dilution of the stock solution with the running buffer when it was needed. Cells were suspended in a balanced phosphate buffered saline (PBS) which was composed of 0.147 M NaH_2PO_4 and 0.2 M Na_2HPO_4 (pH 7.4). All solutions were prepared with doubly distilled water. The above solutions were stored at 4 °C in a refrigerator and were filtered through 0.22 μm polypropylene membrane before its use.

All electropherograms were obtained on a self-assembled CE system. Briefly, a reversible high-voltage power supply (Shandong Normal University Instrument Company, China) provided a variable voltage of 0.0–30.0 kV across the capillary, with the outlet of the capillary at ground potential. A 35 cm fused-silica capillary (25 μm I.D., 375 μm O.D. Yongnian Optical Conductive Fiber Plant, China) was placed between the two buffer reservoirs. The positive pole of high voltage was applied at the injection end, while the negative pole was held at ground potential by a coiled Pt wire placed at the bottom of the reservoir containing the ECD cell. Separations were carried out at an applied voltage of 14.0 kV. The detection cell was shielded in a metal box to reduce external disturbance. A CHI810c Electrochemical Analyzer (Shanghai Chenhua Instrument Company, China) was used to determine the concentration of ascorbic acid with electrochemical detection. ECD was performed with a three-electrode system consisting of a PtNPsME working electrode as the detector, a saturated calomel electrode (SCE) as the reference electrode, and a coiled Pt wire placed at the bottom of the cell as the auxiliary electrode. An inverted biological microscope (Chongqing Optical & Electrical Instrument Co., Ltd. Chongqing, China) and a stereomicroscope (Nanjing Jiangnan Novel Optics Co., Ltd. Jiangsu, China) were used in the experiment. The electrode surface was characterized using an S-4800 ultra-high resolution scanning electron microscopy (Hitachi, Japan).

2.2. Electrode fabrication and modification

The carbon fiber micro-disk electrode (CFME) was fabricated as follows: about 20–30 chops of carbon fibers (*ca.* 6 μm diameter, 7 cm in length) soaking up ethanol were carefully inserted into a fused-silica capillary (*ca.* 250 μm I.D., 375 μm O.D., 3.5 cm in length), and then imbued with ethyl α-cyanoacrylate adhesive. A copper wire's tip (400 μm diameter, 6 cm in length) sharpened by polishing on the abrasive paper was fixed into the other end of the fused-silica capillary and adjusted to connecting the carbon fiber array. The copper wire was also bonded to the fused-silica capillary with ethyl α-cyanoacrylate adhesive. In order to make electric contact well, the carbon fibers around the copper wire were smeared with silver electric adhesive (Sino-platinum Metals Co., Ltd., China) and was dried in the oven at 70 °C for 30 min. The protruding carbon fiber array was cut from the fused-silica capillary. The copper wire's tip was sealed in a glass tube with ethyl α-cyanoacrylate adhesive to protect the glass tube from extrusion and was dried in the air for 24 h [23].

The surface of CFME was carefully polished to a mirror-like surface on a fine sand paper before modification. Then the CFME was sonicated in ethanol and doubly distilled water for 5 min, respectively. Electrodeposition of PtNPs was performed at a constant current potential of −0.30 V (*vs.* SCE) for 20 s in the 0.4 g L^{-1} H_2PtCl_6 and 0.5 M H_2SO_4 solution [24,25]. Finally, the electrode was washed thoroughly with double distilled water for use. When the current of AA was decreased more than 5%, the surface of modified electrode was polished on a fine sand paper to clear the PtNPs and then immobilized with new PtNPs layer by electrodeposition. The area of the basal CFME would change more or less, if different electrodes were used. Therefore, the same electrode was used for all the experiments generally.

2.3. CE procedure and amperometric detection apparatus

The capillaries before use were flushed with 0.2 M NaOH, double distilled water, and the corresponding buffer solution for 20 min, respectively, by means of a syringe. The conditions for the determination of AA: 25 mM PB (pH 7.4) for buffer, 14.0 kV for separation voltage, and 0.70 V for detection potential. An injection voltage of 5.0 kV was applied for 10 s to inject AA solution into the capillary tip. Prior to its use, sample solutions and running buffer were all filtered through a polypropylene filter.

2.4. Cells preparation

Human liver cancer cell lines (HepG2 cells, American Type Culture Collection, Manassas) were cultivated in cells culture media and incubated at 37 °C in a 5% CO_2/95% air humidified incubator